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This project is designed to characterise the sources of phenotypic variability in neurofibromatosis 1 (NF1) by a combination of clinical, statistical, epidemiological, and molecular genetic methods. During the second year of the project, we analysed associations found in clinical and genetic data from the NNFF International Database with logistic regression, multivariate probit models, generalized estimating equations, and multivariate normal models. These sophisticated techniques allowed us to adjust for the complex and pervasive effect of age and other covariates that confounded previous studies. We showed that certain clinical features of NF1 cluster in particular patients and within affected families. We also began our analysis of *NF1* allele-phenotype associations and used the large deletion phenotype in a proof of principle for the method developed for this project. Our results demonstrate the importance of genetic factors in determining not only whether an individual will have NF1 but also what clinical features of the disease will develop.

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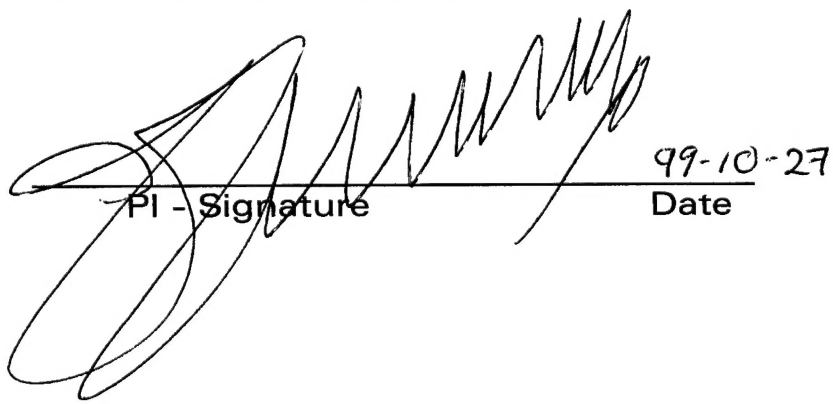

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INTRODUCTION

The natural history of neurofibromatosis 1 (NF1) is incompletely understood. Although NF1 is progressive over the course of an affected individual's life, the rate of progression and the occurrence of serious complications vary greatly. The manifestations of NF1 are extremely variable in different patients of the same age, different affected members of a single family, and even a single affected individual at different times in life. The purpose of this three-year project is to characterise the sources of phenotypic variability in NF1 through a combination of clinical, statistical, epidemiological, and molecular genetic methods. During the project's first year, we used association studies to identify subgroups of NF1 patients in which at least some of the phenotypic variability appears to result from genetic factors, and we set up molecular techniques to identify *NF1* gene mutations. This report covers the second year of the project.

ANNUAL REPORT

Technical objectives 1 and 2: Identification of associations between features of NF1.

Task 1: Identify associations of features within probands by screening the 5000 possible pair-wise associations on both databases.

This task was completed during the first year of the project. (Please see last year's Annual Report; Szudek et al., 1999a [Appendix] for details.)

Task 2: Identify associations of NF1 features between related individuals (e.g. parent-child diads) in both databases.

This task was completed during the first year of the project. (Please see last year's Annual Report and Szudek et al., 1999A [Appendix] for details.)

Task 3: Examine apparent associations (from task 1) in detail for other covariates or confounding factors, using log-linear models for binary traits and stratification for age or other continuous variables.

We found consistent phenotypic associations among various clinical features of NF1 using age-stratified 2 x 2 analyses [Baser et al., 1999 (Appendix); Szudek et al., 1999A (Appendix)], but these phenotypic associations are generally neither strong enough nor characteristic enough to establish prognostically important subgroups of NF1 patients. We, therefore, attempted to extend this analysis by using log-linear models. These efforts were unsatisfactory because log-linear models can only control for age by stratification. We found that differential age adjustment by feature was necessary because each feature of NF1 has a unique relationship with age. Furthermore, stratification requires the establishment of arbitrary age groups that permit only partial adjustment for age.

We, therefore, undertook a series of logistic regressive analyses. These techniques allow age to be transformed to reflect its unique relationship with each feature. In addition, age can be treated as a continuous variable, which increases precision. Logistic regression also permits the simultaneous analysis of associations of more than two features.

We selected 12 especially important or frequent clinical features of NF1 and treated each feature in turn as if it were a disorder that occurs in a population affected with NF1. Using logistic regression, we asked: What is the relationship of the selected (outcome) feature to the other 11 (explanatory) features? (The use of the terms "outcome" and "explanatory" to describe variables is conventional in logistic regression analysis. We are not suggesting a causal relationship between explanatory and outcome features.)

The logit (log of the odds) of each of the 12 features was set as the output variable in a different logistic regression model. Age was controlled as a continuous covariate. Univariate analyses were used to screen for potential main effects. The importance of each of the major explanatory variables was simultaneously assessed in a logistic regression model. Significant terms were used to refit the model, and interaction terms among the explanatory variables were considered.

Fitted logistic regression models always perform in an optimistic manner on the data set used to develop them. Therefore, we developed our models in a random subset of data from half of the 2,797 NF1 probands available to us from the NNFF International Database and tested the best fitting models on data from the subset of probands that was was initially excluded. Forward selection by maximum likelihood estimation was used to identify and quantify significant explanatory features in the developmental subset. Hosmer and Lemeshow goodness-of-fit was calculated for each model. Parameter estimates and goodness-of-fit were then calculated independently in the originally excluded subset, and the results of were compared (Table 1). Models that provided a good fit and had similar parameter estimates in both subsets were considered to be accurate.

Logistic regressive models allow the associations between two or more features to be described as odds ratios. Table 2 shows the odds ratios for the more reliable associations found in the analysis reported in Table 1. Our findings demonstrate that some NF1 patients are far more likely than others to have certain common features of the disorder.

Table 1: Summary of parameter estimates in logistic regressive models of NF1 clinical features.

Output Feature	Explanatory Feature	Initial (Developmental) Subset		Second (Validation) Subset	
		Parameter Estimate	Goodness-of-fit	Parameter Validation	Goodness-of-fit
Freckling	Lisch nodules Neoplasms Female gender	0.2180 0.7484 0.3804	p=0.8753	0.3067 0.6069 0.2022	p=0.7752
Discrete NFs*	Plexiform NFs Lisch nodules	1.2092 0.4642	p=0.8083	0.7870 0.2755	p=0.4085
Plexiform NFs	Discrete NFs Learning disability Scoliosis	1.1301 0.2092 0.6375	p=0.8539	1.1634 0.1556 0.2176	p=0.4064
Lisch nodules	Freckling Discrete NFs Neoplasms	0.3084 0.3280 1.5306	p=0.9235	0.2923 0.5809 0.5445	p=0.5891
Optic glioma	Plexiform NFs Macrocephaly Neoplasms	0.6634 0.4788 1.9652	p=0.4972	0.4987 0.4805 1.9080	p=0.8989
Learning disability	Plexiform NFs Seizures Short stature Male gender	0.4616 0.7962 0.3352 0.3653	p=0.3024	-0.1108 0.0555 0.5622 0.4890	p=0.4897
Seizures	Lisch nodules Learning disability Male gender	-0.5305 0.9719 0.4567	p=0.7901	-0.2391 0.6174 -0.0041	p=0.7381
Pseud-arthrosis	Freckling Discrete NFs Neoplasms	-1.0351 -0.8735 0.7462	p=0.8419	-0.5078 -0.4674 0.3132	p=0.9171
Scoliosis	Plexiform NFs Learning disability	0.6216 0.5504	p=0.0316	-0.6558 0.0290	p=0.0403
Macro-cephaly	Optic glioma Short stature	0.4643 -0.7692	p=0.2853	0.5129 -1.3933	p=0.3710
Short stature	Learning disability Macrocephaly	0.4082 -0.9804	p=0.9960	0.5694 -1.8413	p=0.6211
Neoplasms	Lisch nodules Optic glioma	0.9250 1.8711	p=0.6073	0.4816 1.5751	p=0.0855

* NFs = neurofibromas

Table 2: Summary of associations using logistic regressive models of NF1 clinical features. Results are given as odds ratios with 95% confidence intervals. (Please see Szudek et al., 1999B [Appendix] for methods used in this analysis).

Feature	Associated Features	Odds Ratio (95% C.I.)	
Freckling	Lisch nodules	1.2	(0.8-1.9)
	Neoplasms	2.1	(0.7-6.0)
	Male gender	1.4	(1.1-2.1)
	All three	*3.8*	*(1.2-12)*
Discrete NFs*	Plexiform NFs	3.4	(2.2-4.9)
	Lisch nodules	1.6	(1.1-2.2)
	Both	*5.3*	*(3.3-8.7)*
Plexiform NFs	Discrete NFs	3.1	(2.1-4.6)
	Learning disability	1.2	(0.9-1.7)
	Scoliosis	1.9	(1.3-2.7)
	All three	*7.2*	*(4.0-13)*
Lisch Nodules	Freckling	1.4	(0.9-2.0)
	Discrete NFs	1.4	(1.1-1.9)
	Neoplasms	4.6	(2.1-10)
	All three	*8.7*	*(3.5-22)*
Optic glioma	Plexiform NFs	1.9	(0.9-4.0)
	Macrocephaly	1.6	(0.9-2.9)
	Neoplasms	7.1	(2.8-18)
	All three	*22*	*(5.7-87)*
Pseudarthrosis	No Freckling	2.8	(1.6-4.9)
	No Discrete NFs	2.4	(1.4-4.2)
	Neoplasms	2.1	(0.9-5.2)
	All three	*6.7*	*(3.2-14)*
Neoplasms	Lisch nodules	2.5	(1.1-6.0)
	Optic glioma	6.4	(3.2-13)
	Both	*16.4*	*(5.5-48)*

* NFs = neurofibromas

Task 4: Perform detailed examination of apparent familial associations identified in task 2.

Our previous 2 x 2 analyses demonstrate familial tendencies for the occurrence of clinical features such as optic gliomas, discrete dermal neurofibromas, and Lisch nodules among unselected patients with NF1 [See Szudek et al., 1999A (Appendix)]. Some of these

familial associations are of strikingly similar strength in sib-sib and parent-child comparisons. Other associations, such as those for dermal neurofibromas, are apparent in sibs but not in parent-child pairs, raising the possibility of confounding by age. We, therefore, extended these studies with logistic regression analysis and a multivariate probit model, both of which permit adjustment for the effect of age.

The logistic regression analysis done for features in individual patients (Table 2) set the stage for examining associations of common features among relatives. The method of Liang and Beaty (1991; Liang et al., 1992) is based on a logistic regression model that incorporates effects of individual covariates while measuring familial aggregation of risk as the odds ratios between classes of relatives. We used this method with the software (GEE2) developed by Liang and Beaty to incorporate what we had learned regarding associations between features in probands and simultaneously measure familial aggregation of risk. The associations we found between relatives are summarized in Table 3 as odds ratios with adjustment for the confounding effect of age.

Table 3: Summary of familial associations of NF1 clinical features using logistic regression. 171 NF1 patients and 214 of their affected sibs pairs and 211 affected parents and 289 affected children from the NNFF International Database were analysed using the method and GEE2 software described by Liang and Beaty (1991).

Feature	Odds-ratio (95% C.I.)			
	Sib-Sib		Parent-Child	
Freckling	4.9	(1.8-13)	2.4	(0.7-8.6)
Discrete NFs	6.0	(2.4-15)	4.3	(0.1-170)
Plexiform NFs	1.2	(0.4-3.8)	1.7	(0.7-4.2)
Lisch nodules	13.0	(3.7-44)	3.9	(1.1-15)
Learning disability	3.6	(1.8-7.6)	1.0	(0.5-1.9)
Scoliosis	1.8	(0.5-6.5)	1.0	(0.3-3.0)
Macrocephaly	5.3	(1.5-19)	3.5	(1.0-13)
Short stature	4.0	(1.1-14)	5.3	(1.7-16)
Neoplasms	21.8	(1.5-310)	20.9	(5.3-82)

Technical objective 5: Determine the contribution of genetic and non-genetic factors to the presence or absence of certain NF1 traits.

Task 5: For traits showing familial aggregation (from task 2), partition variance by using various techniques including logistic regressive modelling and multivariate normal methods.

Some of the genetic associations we observed (Table 3) are surprisingly large for a condition in which intrafamilial discordance, rather than resemblance, is thought to be the rule [Borberg 1951; Sørensen et al. 1986a, 1986b; Huson et al. 1989a, 1989b; Samuelsson and Samuelsson 1989; Riccardi 1992; Zöller et al. 1995, 1997a, 1997b; Carey and Viskochil, 1999]. To investigate these striking familial associations further, we calculated correlation coefficients and standard errors for several clinical features among sibs, parent-child pairs, and second-degree relatives who are affected with NF1. Binary data from the NNFF International Database were analysed using a multivariate probit model with adjustment for age. Ordinal data from 147 individuals with NF1 in 64 families from Dr. Vincent Riccardi's NF Institute Database were analysed with a transformed multivariate normal model and adjustment for age. These analyses were done in collaboration with Dr. Harry Joe, who has described these techniques in detail [Joe, 1997].

Table 4 summarizes the correlations we calculated and compares our findings with those of the only previous study in which similar correlations were reported [Easton et al., 1993; Easton, 1998]. These data must be interpreted cautiously because several of the estimated correlations have large standard errors. This is especially true for second degree relatives. Easton et al. (1993) did not report standard errors for their estimates, but their sample sizes are similar to ours.

Various clinical features of NF1 tend to appear at particular times of life, but most of these times are in the first 25 years, the period that encompasses the difference in age between a parent and his or her offspring. This means that many important features like cutaneous neurofibromas are present in most parents with NF1 but absent in most affected young children. In such circumstances, knowing that a parent is affected or that a child is unaffected is unlikely to reveal much about underlying genetic factors that promote the change of status from "absent" to "present". Statistical methods of adjusting for the effect of age cannot adequately deal with this issue in cross-sectional binary data. Age adjustment is much more effective with quantitative data if a greater number of lesions reflects a greater propensity to develop the feature. This may account for the higher parent-child correlation seen when we treated cutaneous neurofibromas as quantitative (ordinal) variable rather than as a binary variable (Table 4). More precise age adjustment may also account for the fact that we found a substantially higher correlation for cutaneous neurofibromas in sibs than Easton et al. (1993) did. Easton treated age as a categorical variable with 10-year age increments before adjusting for age by linear regression. We treated age as a continuous covariate in our analysis.

Table 4: Correlation coefficients (and standard errors) for several clinical features among affected relatives with NF1. See Easton et al. (1993) for methods used in that study. Our analyses were performed in collaboration with Dr. Harry Joe using binary data from the NNFF International Database and ordinal data from 147 individuals with NF1 in 64 families collected by Dr. Vincent Riccardi. Binary data were analyzed using a multivariate probit model with adjustment for age. Ordinal data were analyzed with a transformed multivariate normal distribution and adjustment for age.

Clinical Feature	Variable Type	Correlation (S.E.)			Reference
		Sib-Sib	Parent-Child	2° Relatives	
Lisch nodules	Binary	.74 (.09)	.79 (.10)	-.06 (.43)	Current project
Dermal neurofibromas	Binary	.59 (.11)	.09 (.13)	.20 (.25)	Current project
Cutaneous neurofibromas	Ordinal*	.75 (.09)	.24 (.12)		Current project
Cutaneous neurofibromas	Ordinal*	0.15	0.19	0.05	Easton et al., 1993
Café-au-lait spots	Integer	0.34	0.37	0.17	Easton et al., 1993
Head circumference	Continuous	0.59	-0.19	0.15	Easton et al., 1993

* Dermal neurofibromas were treated as an ordinal variable in both of these analyses, but the classification was different. In Easton et al. (1993), the classes were based on approximate counts (0, 1-9, 10-99, 100-499, or >500). In our studies, the presence or absence of tumours was determined in each of 10 different regions of the body, and patients were scored as 0-10 to reflect the number of regions that contained one or more cutaneous neurofibromas.

The analyses shown in Tables 3 and 4 support the importance of genetic factors in determining the phenotypic expression of NF1. Our findings also suggest that the nature of these factors differs for different features. For example, we found very strong correlations for the occurrence of Lisch nodules in both parent-child pairs and sib-sib pairs. No correlation was found among second-degree relatives for this feature. If this pattern of correlations holds up with additional studies, it would suggest the involvement of modifying

genes in the occurrence of Lisch nodules. In contrast, both our binary and ordinal data for cutaneous neurofibromas show a much stronger correlation among sibs than between parents and their children. This pattern suggests a possible influence of the normal NF1 allele on the phenotype.

Technical objective 6: Identify allele-phenotype correlations between "familial phenotype" and allele type.

Task 1a: Set up techniques for strategic screening process for identification of constitutional mutations of NF1.

Dr. Viskochil's laboratory has implemented a strategy to screen for germ-line *NF1* mutations in individuals with unusual familial clinical presentations. The approach combines exon genotype analysis, fluorescence in situ hybridization (FISH), cDNA synthesis with restriction enzyme digestion mapping, and size-shift analysis of exons amplified from genomic DNA.

Our primary emphasis has been on genomic DNA screening by size-shift analysis to identify small deletions and insertions. We have comprehensively screened 30 individuals with NF1 using the size-shift protocol and detected 10 mutations. We have implemented and validated the other components of this multistep mutation screening protocol as well. We prepared *NF1* cDNA and PCR amplicons of five cDNA segments for the *in vitro* translation test. We can amplify cDNA segments that are over 2 kb in length. We have generated enough material to carry out restriction enzyme digestions using agarose gels and ethidium bromide staining as a detection method in proof-of-principle analyses. We have also successfully applied MS-PCR to identify heterozygous individuals using genomic DNA. Samples that are not heterozygous have been screened by fluorescence *in situ* hybridization (FISH) to identify four individuals with whole-gene deletions.

Identification of some *NF1* mutant alleles remains a challenge. This is due in part to the large size of the gene and the lack of mutational hot spots. Techniques used by other workers to identify *NF1* mutations include heteroduplex analysis, single-strand conformation polymorphism analysis [Upadhyaya et al. 1995; Abernathy et al. 1997], Southern analysis, denaturing gradient gel electrophoresis, chemical cleavage of mismatched sites, and FISH (for large deletions). These DNA-based tests have generally identified mutations in no more than 50% of NF1 patients, although many studies have only screened part of the gene, usually focussing on the *NF1* domain that has Ras-GTPase activating activity. The most effective single method of *NF1* mutation detection described to date is the protein truncation test (PTT), an *in vitro* translation test that identified authentic mutations in over 2/3 of patients in two small series [Heim et al.1995; Park and Pivnick 1998]. The sensitivity and specificity of this test have not yet been demonstrated in clinical practice.

Task 7 (Vancouver): Identify 40 patients for mutation identification from familial phenotypes identified in task 2. Contact contributing centres and obtain blood samples.

and

Task 2a (Salt Lake City): Identify mutations in these 40 NF1 patients.

In our last annual report, we listed 17 possible phenotypic subgroups that were under consideration for case-control studies of allele-phenotype associations. From this list, we subsequently chose the following subgroups as the ones that are most promising for these studies:

- "Large deletion phenotype"
- Malignant peripheral nerve sheath tumours
- NF1 vasculopathy
- Optic glioma and other central nervous system gliomas
- Multiple subcutaneous and paraspinal neurofibromas
- Late-diagnosed NF1, an initially mild phenotype

These sub-groups are discussed below:

Large deletion phenotype: We began with the large deletion phenotype, a subgroup recognized because of its association with a particular class of mutation [*e.g.* Wu et al, 1995; Tonsgard et al, 1997; Upadhyaya et al, 1998] to evaluate our approach in principle. We selected 11 patients with features of this phenotype from the NNFF International Database without knowledge of their *NF1* genotype. Mutation analysis has been performed on four of these patients, and three were found to have large *NF1* deletions detectable by FISH. This finding provides reassurance that our method for selecting patients for genotype-phenotype studies is valid.

Malignant peripheral nerve sheath tumours: Several familial cases of malignant peripheral nerve sheath tumours have been reported. [e.g. Schearer et al. 1994; Poyhonen et al. 1997; Gareth Evans, personal communciation] Although one might not expect an allele-phenotype correlation to occur for a feature that requires a second, presumably random, mutational "hit" to develop, unequivocal allele-phenotype associations have been observed for tumour-related phenotypes in NF2 [Evans et al, 1998; Kluwe et al, 1998], Li-Fraumeni [Birch et al, 1998] and von Hippel-Lindau syndrome [Crossey et al, 1994; Maher et al, 1996].

NF1 vasculopathy: Mutations of the *NF1* gene can cause severe vasculopathy The process most often affects arterial vessels, and the systemic circulation is affected much more often than the pulmonary arteries. The lesions vary from stenosis to aneurysmal dilation. The condition is sometimes progressive, with lesions becoming more severe with time or recurring after effective treatment [Pentecost et al. 1981; Kurien et al. 1997]. Most NF1 patients with vasculopathy have involvement of multiple vessels [Wertelecki et al. 1982; Zachos et al, 1997], an observation that suggests a generalized propensity to developing vascular lesions. Familial occurrence of hypertension and its complications in association

with NF1 has been reported [Bertrand et al. 1992].

Optic glioma and other central nervous system gliomas: NF1 patients with optic gliomas appear to have an unusually high frequency of other central nervous system gliomas [Kuenzle et al. 1994; Friedman and Birch, 1997; Bilaniuk et al. 1997]. The association between optic and other central nervous system gliomas does not result from observation bias, radiation treatment for optic glioma, or confounding by age [Friedman and Birch, 1997]. These findings suggest that NF1 patients with optic and other CNS gliomas have a genetic predisposition to develop astrocytic tumours.

Multiple subcutaneous and paraspinal neurofibromas: Our collaborator, Dr. Vincent Riccardi, has identified a small subgroup of NF1 patients who have extensive deep nodular and paraspinal plexiform neurofibromas [Riccardi, 1992, pp. 188-190; and personal communication]. Such patients may have severely disabling or lethal complications related to neurological compromise or compression of cervical or mediastinal structures [Riccardi, 1992, *e.g.* cases NF78-40-1 and NF86-723-1]. Although children with NF1 often have diffuse plexiform neurofibromas, nodular subcutaneous neurofibromas are uncommon in childhood. In contrast, the deep nodular and paraspinal plexiform neurofibromas in this subgroup are often apparent in childhood.

Late-diagnosed NF1, an initially mild phenotype: Most children with NF1 unequivocally meet the NIH Diagnostic Criteria [Cnossen et al, 1997, 1998; DeBella et al, in press] by 8 years of age. However, there is a small subgroup who have multiple café au lait spots but no other characteristic features of NF1 until age 16 or later, and a group of adults with café-au-lait and a very few cutaneous neurofibromas as their only symptoms. We have identified a number of families in which multiple affected relatives have this mild NF1 phenotype. We have requested blood specimens on members of 7 such families.

We have only obtained blood samples for molecular analysis from patients with this mild phenotype or with the "large deletion phenotype". Although we have requested samples on patients with some of the other selected phenotypes, obtaining these samples has been difficult. Part of the difficulty results from our inability to approach patients directly because of the confidentiality constraints built into this project and the NNFF International Database. This means that we must go through physicians at each local contributing centre to obtain blood samples on their patients. These collaborating physicians are not paid for their contributions to the NNFF International Database, but we have recently instituted a system to compensate them for the time spent on this project. We have also hired a part-time assistant to facilitate the interactions necessary to obtain blood specimens from collaborating investigators throughout the world.

Task 6: Review all cases with "familial phenotypes" in the NF1 Genetic Analysis Consortium Database and the published literature, looking for common mutations or mutation types.

This has proven to be an unrewarding task. Neither the NF1 Genetic Analysis Consortium Database nor the published literature provides detailed clinical descriptions on most patients on whom germline *NF1* mutations have been identified. The only phenotypic subgroup listed in the previous section for which we were able to identify a common mutation or mutation type was the "large deletion phenotype".

KEY RESEARCH ACCOMPLISHMENTS
(Year 2)

- Demonstration of associations among clinical features in NF1 patients using logistic regression to control for the confounding effect of age. (See Tables 1 and 2).

- Demonstration of associations of NF1 clinical features in family members using logistic regression and multivariate probit models to control for the confounding effect of age. (See Table 3).

- Demonstration of strong correlations of NF1 clinical features among affected relatives with multivariate probit and normal models (See Table 4).

- Proof of principle for analysis of *NF1* allele-phenotype associations.

REPORTABLE OUTCOMES
(Year 2)

1. **Manuscripts, abstracts and presentations**: (All abstract, reprints, and unpublished manuscripts are included in the appendix).

Papers published in peer-reviewed scientific journals

Baser ME, Birch PH, Evans GR, Friedman JM: **Association of superficial plexiform and paraspinal neurofibromas in neurofibromatosis 1.** *Neurology* 1999 Apr 22;52(7):1519-20

Stevenson DA, Birch PH, Friedman JM, Viskochil DH, Balestrazzi P, Boni S, Buske A, Korf BR, Niimura M, Pivnick EK, Schorry EK, Short MP, Tenconi R, Tonsgard JH, Carey JC: **Descriptive analysis of tibial pseudarthrosis in patients with neurofibromatosis 1.** *Am J Med Genet* 1999 Jun 11;84(5):413-9

Papers accepted for publication in peer-reviewed scientific journals

DeBella K, Szudek J, Friedman JM: **Use of the NIH Criteria for Diagnosis of NF1 in Children** [Accepted for publication: *Pediatrics*]

Manuscripts submitted for publication (meeting presentations as noted*)

DeBella K, Poskitt K, Szudek J, Friedman JM: **Use of unidentified bright objects on MRI for diagnosis of neurofibromatosis 1 in children.** [Submitted for publication: *Neurology*]
*Oral presentation: American Society of Human Genetics, October 1999
Published abstract: *Am J Hum Genet* 65(3) A36 Supplement, 1999

Szudek J, Birch P, Friedman JM: **Analysis of growth in neurofibromatosis 1 (NF1)** [Submitted for publication: *J Med Genet*]

Szudek J, Birch P, Friedman JM: **Growth curves for children with neurofibromatosis 1 (NF1)** [Submitted for publication: *J Med Genet*]

Szudek J, Birch P, Riccardi VM, Evans DG, Friedman JM: **Associations between clinical features of neurofibromatosis 1 (NF1)** [Submitted for publication: *Am J Hum Genet*]

Manuscripts in preparation (meeting presentations as noted*)

Lin AE, Birch PH, Korf BR, Schneider G, Tenconi R, Niimura M, Poyhonen M, Armfield-Uhas, K, Sigorini M, Wolkenstein P, Pivnik E, Lawrence M, Friedman JM: **Cardiovascular Malformations and other Cardiac Abnormalities in Neurofibromatosis 1 (NF1)** [To be submitted to *Am J Med Genet*]
*Oral presentation: National Neurofibromatosis Foundation Annual Symposium, October 1999.

Rasmussen SA, Yang QH, Friedman JM: **Mortality associated with neurofibromatosis 1 in the United States from 1983 to 1995: an analysis using data from death certificates.** [To be submitted to *Am J Hum Genet*]
*Oral presentation: American Society of Human Genetics, October 1999
Published abstract: *Am J Hum Genet* 65(3) A49 Supplement, 1999

Szudek J, Joe H, Friedman JM: **Logistic Regressive Models of Neurofibromatosis 1 (NF1) Features.**
*Oral presentation: American Society of Human Genetics, October 1999
*Poster presentation: 8th European Neurofibromatosis Symposium, Ulm, Germany, September 1999
Published abstract: *Am J Hum Genet* 65(3) A36 Supplement, 1999

2. Informatics resources:

The NNFF International Database was converted from a DOS-based Clipper database to MS-Access, and facilities were developed to permit data entry via the World Wide Web or through scannable paper forms. These systems have all been fully tested and implemented; they have also been verified as Y2K compliant. Our new data entry facilities enable NF centres throughout the world to participate in the NNFF International Database more easily. Data entry over the Web can be used by any kind of computer with Internet access and avoids the difficulties we have had in the past with potential contributors who use Macintosh computers or are unfamiliar with DOS applications. The scannable forms do not require access to a computer at all. Moreover, they can be translated into any language without the need to reprogram the data entry system.

The NF Institute Database, which contains detailed clinical information on the large series of neurofibromatosis patients seen personally by Dr. Vincent Riccardi, was originally compiled on paper forms. Some of the information from each patient's first and most recent visits were recorded in an electronic database several years ago. We have recently transferred these data to MS-Access and have entered additional quantitative and family data from the paper forms. This new database has provided crucial information for our studies of familial aggregation of various traits in NF1 patients.

3. Employment and training

Employment and training has been provided through this project to 2 U.S. students, 3 Canadian students, and one international student. Two of these individuals are graduate students in medical genetics, two are graduate students in statistics, and three are undergraduate or immediate post-baccalaureate students who are now pursuing graduate or medical degrees. The training environment in our laboratory has been substantially enhanced by a fruitful collaboration Professor Harry Joe of the University of British Columbia Statistics Department.

4. Matching Grants

One of our students, a US citizen, is being partially supported by a training grant from the Government of British Columbia, Department of Science and Technology.

CONCLUSIONS

Summary of Results To Date

This project is designed to characterise the sources of phenotypic variability in NF1 by a combination of clinical, statistical, epidemiological, and molecular genetic methods. During the project's second year, we extended our previous analyses of clinical associations with sophisticated statistical techniques that permit adjustment for the complex and pervasive effect of age. We demonstrated associations among clinical features in NF1 patients (Tables 1 and 2), associations of NF1 clinical features in family members (Table 3), and strong correlations of NF1 clinical features among affected relatives (Table 4). We also began our analysis of *NF1* allele-phenotype associations by using the large deletion phenotype in a proof of principle for our methods.

Importance and Implications

NF1 is one of the most common genetic disorders. It is extremely variable clinically, with features ranging from benign café-au-lait spots or iris Lisch nodules to serious or life-threatening manifestations such as dysplastic scoliosis, nerve root compression, and malignancy. NF1 is progressive, but the rate of progression and the occurrence of serious complications vary among patients and within affected members of a family, all of whom have the same mutation of the *NF1* gene. This phenotypic variability and the resulting uncertainty about the disorder's future clinical course are a great burden to affected families. Moreover, the extensive variability of NF1 complicates clinical management, limits the value of genetic counselling, confounds treatments studies, and has precluded most attempts to establish genotype-phenotype correlations.

Understanding the basis for the marked phenotypic variability in NF1 is essential to understanding its pathogenesis. Our studies demonstrate that genetic factors are involved in determining what clinical features of the disease will develop in a particular patient with NF1.

Changes Recommended To Address the Problem Better

The most important lesson we have learned regarding application of our statistical and epidemiological methods to NF1 is how complex and pervasive age-related confounding is. We found, for example, that age adjustment has an inconsistent effect on the familial associations we observed for binary (presence/absence) variables. Statistical methods of adjusting for the effect of age cannot adequately deal with this issue in cross-sectional binary data.

We do not believe that the pervasiveness of age confounding in NF1 has been appreciated

fully by investigators in this field. For example, Easton and his associates [1993; Easton, 1998] reported intra-familial correlations in the number of café-au-lait macules and cutaneous neurofibromas as well as associations between the presence or absence of optic gliomas, scoliosis and seizures among relatives with NF1. The strength of some of these associations appeared to decrease with decreasing genetic similarity, a finding that suggests the involvement of modifying genes. However, Easton et al. evaluated their binary trait data by inspection without adjustment for the profound effects of age, so it is difficult to interpret their findings.

There are three general approaches to dealing with this problem. The first is to use techniques such as logistic regression, multivariate probit models, and multivariate normal models that allow us to adjust for the effect of age as a continuous variable. We have done this, but it is not always adequate, as discussed above.

A second approach is to employ quantitative variables, which are more informative and permit more precise age adjustment than binary variables. This is one of the reasons we used quantitative data to estimate the familial correlations presented in Table 4. Unfortunately, available data sets contain only a limited amount of reliable quantitative information on NF1 patients.

A third approach is to use longitudinal rather than cross-sectional data, and we shall pursue this approach during the third year of our project. Longitudinal data also provide an important source of quantitative clinical variables because age at onset of particular symptoms can be determined with more precision. Our current analyses, which are based on cross-sectional data, only estimate the risks of two subgroups in comparison to each other Longitudinal data are necessary to calculate absolute risks for the development of particular complications in specific patient subgroups (Tables 2 and 3).

To obtain more quantitative and longitudinal data for these studies, we have established a collaboration with Dr. Gareth Evans, whose NF1 database from Manchester, England, includes clinical information on 270 probands, 140 affected parent-child pairs and 77 affected sib pairs with NF1. There is no overlap between this database and the others we have been using. We have also devoted substantial effort to converting Dr. Vincent Riccardi's NF Institute Database from paper files to a fully computerized form for use in this project.

The confounding effect of age, especially with binary variables, prevents us from completing Task 5 (partitioning the variance of traits showing familial aggregation) as planned in our original Statement of Work. However, the use of quantitative variables with careful adjustment for age and covariates has enabled us to calculate much stronger correlation coefficients than have ever been observed before for features among relatives with NF1 (Table 4). We shall extend these studies in the third year of the project to produce more reliable estimates of the contribution of genetic factors to the variability of quantitative traits in NF1.

The most serious logistical problem we have encountered is obtaining blood samples on patients with selected phenotypes for our genotype-phenotype studies. These samples must be requested through local NF Clinic directors who voluntarily contribute data to the NNFF International Database. We recently hired a part-time assistant who will devote her time to this aspect of the project. We are also making arrangements with individual NF Clinic directors to facilitate their efforts to obtain samples for this study.

So What?

Our results help to define subgroups of patients with NF1 who are especially likely (or unlikely) to develop serious complications of the disorder. This will permit screening efforts to be focussed more effectively on those at greatest risk.

Of even greater long-term benefit is the insight these studies provide into the extensive clinical variability that characterizes NF1. Understanding the reasons for this clinical variability is necessary to understand how mutations cause NF1 and to provide new approaches to prevention and treatment.

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APPENDIX

Papers published in peer-reviewed scientific journals

Baser ME, Birch PH, Evans GR, Friedman JM
Association of superficial plexiform and paraspinal neurofibromas in neurofibromatosis 1.
Neurology 1999 Apr 22;52(7):1519-20

Stevenson DA, Birch PH, Friedman JM, Viskochil DH, Balestrazzi P, Boni S, Buske A, Korf BR, Niimura M, Pivnick EK, Schorry EK, Short MP, Tenconi R, Tonsgard JH, Carey JC
Descriptive analysis of tibial pseudarthrosis in patients with neurofibromatosis 1.
Am J Med Genet 1999 Jun 11;84(5):413-9

Papers accepted for publication in peer-reviewed scientific journals

DeBella K, Szudek J, Friedman JM
Use of the NIH Criteria for Diagnosis of NF1 in Children
[Accepted for publication: *Pediatrics*]

Manuscripts submitted for publication (meeting presentations as noted)

DeBella K, Poskitt K, Szudek J, Friedman JM
Use of unidentified bright objects on MRI for diagnosis of neurofibromatosis 1 in children.
Oral presentation, American Society of Human Genetics, October 1999
[Published abstract: *Am J Hum Genet* 65(3) A36 Supplement, 1999]
[Paper submitted for publication: *Neurology*]

Szudek J, Birch P, Friedman JM
Analysis of growth in neurofibromatosis 1 (NF1)
[Submitted for publication: *J Med Genet*]

Szudek J, Birch P, Friedman JM
Growth curves for children with neurofibromatosis 1 (NF1)
[Submitted for publication: *J Med Genet*]

Szudek J, Birch P, Riccardi VM, Evans DG, Friedman JM
Associations between clinical features of neurofibromatosis 1 (NF1)
[Submitted for publication: *Am J Hum Genet*]1999a.

Manuscripts in preparation (meeting presentations as noted)

Lin AE, Birch PH, Korf BR, Schneider G, Tenconi R, Niimura M, Poyhonen M, Armfield-Uhas, K, Sigorini M, Wolkenstein P, Pivnik E, Lawrence M,Friedman JM
Cardiovascular Malformations and other Cardiac Abnormalities in Neurofibromatosis 1 (NF1)
Oral presentation, National Neurofibromatosis Foundation Annual Symposium, Oct 1999.
[Manuscript completed, pending author's signatures. To be submitted to *Am J Med Genet*]

Rasmussen SA, Yang QH, Friedman JM
Mortality associated with neurofibromatosis 1 in the United States from 1983 to 1995: an analysis using data from death certificates.
Oral presentation, American Society of Human Genetics, October 1999
[Published abstract: *Am J Hum Genet* 65(3) A49 Supplement, 1999]

Szudek J, Joe H, Friedman JM
Logistic Regressive Models of Neurofibromatosis 1 (NF1) Features
Oral presentation, American Society of Human Genetics, October 1999
Poster presentation, 8th European Neurofibromatosis Symposium, Ulm, Germany, September 1999
[Published abstract: *Am J Hum Genet* 65(3) A36 Supplement, 1999b]

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Association of superficial plexiform and paraspinal neurofibromas in neurofibromatosis 1 (NF1)

To the Editor:

Tonsgard et al. [1] performed CT imaging in 91 adult ($\geq$16 years) NF1 patients; paraspinal neurofibromas occurred in 18.7% of patients in the chest region and in 25.3% of patients in the abdominal or pelvic region. The majority of patients were asymptomatic.

We wondered whether the presence of plexiform neurofibromas that are apparent on the surface of the body could be used to identify NF1 patients who are at high risk of having paraspinal neurofibromas as well. We therefore examined the association between NF1 superficial plexiform and paraspinal neurofibromas in both the National Neurofibromatosis Foundation International Database (NNFFID; 2,638 patients) [2] [3] and an English series (288 patients).

Superficial plexiform neurofibromas occurred in 23.2% of NNFFID patients and 24.0% of English patients. Paraspinal neurofibromas occurred in 1.7% of NNFFID patients and 4.2% of English patients. The low frequency of paraspinal tumors is probably due to failure to identify most asymptomatic tumors, compounded by a pediatric ascertainment bias in the NNFFID (61.8% of patients were <16 years old).

Using the NNFFID data, we age- and sex-matched NF1 patients with plexiform neurofibromas 1:2 with NF1 patients who lacked plexiform neurofibromas. In all age groups, 3.3% of patients with plexiform neurofibromas had paraspinal neurofibromas, compared to 1.3% of patients without plexiform neurofibromas (odds ratio [OR] = 2.6; 95% confidence interval [CI] = 1.4 to 4.9). In patients <16 years old, 3.5% of patients with plexiform neurofibromas had paraspinal neurofibromas, compared to 0.7% of patients without plexiform neurofibromas (OR = 5.4; 95% CI = 2.0 to 15.2).

In the English series, 8.7% of patients with plexiform neurofibromas had paraspinal neurofibromas, compared to 2.7% of patients without plexiform neurofibromas (OR = 3.4; 95% CI = 0.9 to 13.1). There were too few young patients to conduct an age-stratified analysis. In both the NNFFID and the English series, no

other abnormalities were significantly overrepresented in patients with plexiform

neurofibromas.

Although the biological basis for an association between surface and paraspinal neurofibromas is unknown, there are several possibilities (which are neither mutually exclusive nor the only ones). Easton et al. [4] modeled NF1 traits to determine if the variable expression of NF1 had an inherited component. They concluded that NF1 trait-specific phenotypes (including plexiform neurofibromas) were largely determined by genotypes at modifying loci, although a precise model could not be specified. Some patients may have a form of NF1 that highly predisposes to the development of plexiform neurofibromas involving both superficial structures and spinal nerves and ganglia. These patients might have a higher-than-expected paraspinal and superficial plexiform tumor count based on age, analagous to *NF1* deletions and a large number of neurofibromas based on age. [5] In this case, the lesions would not necessarily occur in the same area of the spine. Paraspinal neurofibromas might secrete a factor that causes overgrowth of susceptible tissues in a paracrine fashion, so that a plexiform tumor that lies adjacent to a spinal nerve root or ganglion might promote the proliferation of the cells to become a paraspinal tumor. This mechanism would produce surface and paraspinal plexiform neurofibromas that are spacially contiguous. Our data on tumor location are not detailed enough to test the last two hypotheses, but future research may do so.

Although we observed an association between surface plexiform and paraspinal neurofibromas, the sensitivity and specificity were not high enough for the association to be used clinically to identify individual patients at high risk of paraspinal neurofibromas. Further research is necessary to define the natural history of paraspinal neurofibromas in NF1 patients and to determine the most effective means of identifying patients who are likely to develop serious complications from these lesions.

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American Journal of Medical Genetics 84:413–419 (1999)

Descriptive Analysis of Tibial Pseudarthrosis in Patients With Neurofibromatosis 1

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Five percent of individuals with neurofibromatosis type 1 (NF1) present with congenital long bone pseudarthrosis (PA). In large series, 50–80% of patients with congenital long bone PA also have NF1. Very little information exists on the natural history and pathogenesis of PA in NF1. This report is a descriptive analysis of a large series of patients with NF1 and tibial bowing or PA. Study A is a case-control study using the National Neurofibromatosis Foundation International Database (NNFFID). Eighty-five patients with PA were compared to a control group from the same database. There was a statistically significant male predominance of NF1 cases with PA (54 males to 31 females), compared to controls (85 males to 87 females) ($\chi^2 = 4.0$, $P = 0.046$, using a two-tailed test with Yates' correction). There was no significant difference in the clinical presentation of NF1 manifestations in NF1 patients with PA than in NF1 patients without PA. Of the affected individuals with PA, there were 24 de novo cases and 21 familial cases (9 through maternal and 12 through paternal inheritance). Questions that could not be answered by Study A were addressed by a partially overlapping case-series report, Study B, in which data on 75 cases ascertained through questionnaires completed by NF center directors were collected. From Study B we determined that half of the patients who had a fracture sustained it before age 2, and approximately 16% of the pseudarthrosis patients had an amputation. Our data indicate a male predominance and no parent-of-origin effect. Male gender may be a susceptibility factor for pseudarthrosis in NF1. Am. J. Med. Genet. 84:413–419, 1999. © 1999 Wiley-Liss, Inc.

KEY WORDS: neurofibromatosis type 1; pseudarthrosis; tibial bowing; bone dysplasia

INTRODUCTION

Neurofibromatosis type 1 (NF1) is one of the most common genetic disorders of childhood. Among the many associated manifestations of NF1 is the orthopedic complication of long bone pseudarthrosis, usually tibial pseudarthrosis. Ducroquet [1937] first observed that tibial pseudarthrosis is related to NF1. Approximately 5% of patients with NF1, from NF clinics that reported to an international database, have this osseous dysplasia [Friedman and Birch, 1997] and about 50–80% of all reported cases of pseudarthrosis have NF1 [Gilbert and Brockman, 1995; Morrissy et al., 1981; Sofield, 1971]. The literature indicates that NF1 patients with this condition initially present with an-

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terolateral bowing of the long bone [Crawford and Bagamery, 1986; Rudicel, 1987]. Therefore, this type of bowing can be easily distinguished from the mild lateral bowing commonly present in the pediatric population. While the term congenital is usually applied, most patients with or without NF1 present with bowing later than birth, usually in the first year of life. Very little information exists on the natural history and pathogenesis of NF1-related pseudarthrosis, a biologically intriguing and medically challenging condition.

The biologic basis of long bone bowing and pseudarthrosis in NF1 is not known. The bony defects are primary dysplasias and presumably not secondary responses to neurofibromas. While most of the medical manifestations of NF1 involve cells derived from the neural crest, there is no easy explanation for the mesodermally derived osseous defect in NF1.

Our understanding of the natural history of NF1 patients with long bone dysplasia and pseudarthrosis is limited. Such information, if it were available, would be helpful for the management and counseling of these patients. There is no detailed large series study on the natural history of pseudarthrosis in NF1 patients. Some of the questions regarding the biology and natural history of PA in NF1 can be addressed using the National Neurofibromatosis Foundation International Database (NNFFID) [Friedman et al., 1993] and a network of clinic directors. Three specific questions are posed: 1) Are the demographics, clinical manifestations, and developmental history of patients with NF1 different in patients with and without pseudarthrosis? 2) Is there an affected parent-of-origin effect? 3) What is the natural history of tibial bowing and PA in NF1 (i.e., age of onset, age at fracture, number of operations, and rate of amputation)?

METHODS

Information on patients with NF1 is available through the NNFFID. Contributors to the database contribute standard information regarding their NF patients. These data were reviewed and evaluated. In addition, we investigated several natural history questions that are not addressed by the database. A questionnaire was designed to collect information on the natural history of NF1 patients with long bone dysplasia. Thus, this investigation includes two different methods for ascertainment of data. The two components of the study are designated Study A (using the NNFFID) and Study B (using a questionnaire sent to NF clinic directors).

Patient Selection

Study A, the database component, is a case-control study using the NNFFID [Friedman et al., 1993]. This database is a system for collecting comprehensive information on the clinical manifestations of NF. The database currently contains detailed clinical information on individuals contributed by 25 clinics throughout the world. Information is collated in a central database. Confidentiality is maintained by identifying patients by a database number. Local clinics can identify individual patients by linking this database number to the patient's name. Cases with osseous dysplasia of the tibia and/or fibula were selected from the database.

Of the 1,479 unrelated individuals with NF1 included in the database at the time of this analysis, 85 individuals or 5.7% had long bone bowing or pseudarthrosis. Of these, 52 or 3.5% of reported NF1 patients are described as having pseudarthrosis, with the remainder having long bone bowing.

Study B, the questionnaire component, is a case series intended to obtain information on the natural history of pseudarthrosis that was not available in the database. Patient selection consisted of personally contacting NF centers around the world to obtain more specific data on their patients with tibial bowing and pseudarthrosis. Invitations to contribute information on patients were sent to 21 NF centers, of which 10 responded with completed questionnaires.

For confidentiality, centers were asked to use identification numbers instead of names. Thirty patients identified in this survey had identification numbers identical to those of Study A. Using this approach demographic, genetic, and clinical data on 75 patients from various international NF clinics were collected. The data were evaluated for aspects of disease presentation and the presence or absence of certain variables relating to NF1 and pseudarthrosis.

Diagnosis Criteria

Cases with long bone dysplasia who did not meet the NIH criteria for NF1 [Stumpf et al., 1988], were excluded. NIH criteria for the diagnosis of NF1 include at least two of the following findings: six or more café-au-lait spots greater than 5 mm in diameter in prepubertal subjects and greater than 15 mm in postpubertal subjects, two or more neurofibromas or one plexiform neurofibroma, intertriginous freckling, distinctive bone lesions (sphenoid wing dysplasia or pseudarthrosis), two or more Lisch nodules, an optic glioma, or a first-degree relative diagnosed with NF1.

One problem encountered was the difficulty of defining pseudarthrosis and the wide spectrum of associated osseous abnormalities. The classic presentation is tibial bowing (Fig. 1) leading to fracture that results in non-union. However, the spectrum of severity includes simple anterolateral bowing with cortical thickening, hairline fractures, fracture with and without healing after varying times, fibular involvement, amputations, bone grafts, and surgeries before fracture. Various classifications of pseudarthrosis have been published, including Boyd's classification and the more recent classification system by Crawford. None has been widely adopted [Andersen, 1973, 1976a, 1976b; Bassett et al., 1980; Boyd and Sage, 1958; Crawford, 1986; Masserman et al., 1974; McFarland, 1951; Morrissy, 1981; Rathgeb et al., 1974; Sofield, 1971]. The clinician at each referring center determined if cases had any of the above mentioned forms of osseous dysplasia of the tibia and/or fibula. Such cases were included in both Studies A and B.

For this study, two groups were delineated: Group 1 and Group 2. Cases with only simple anterolateral bowing of the tibia or fibula were placed in Group 1.

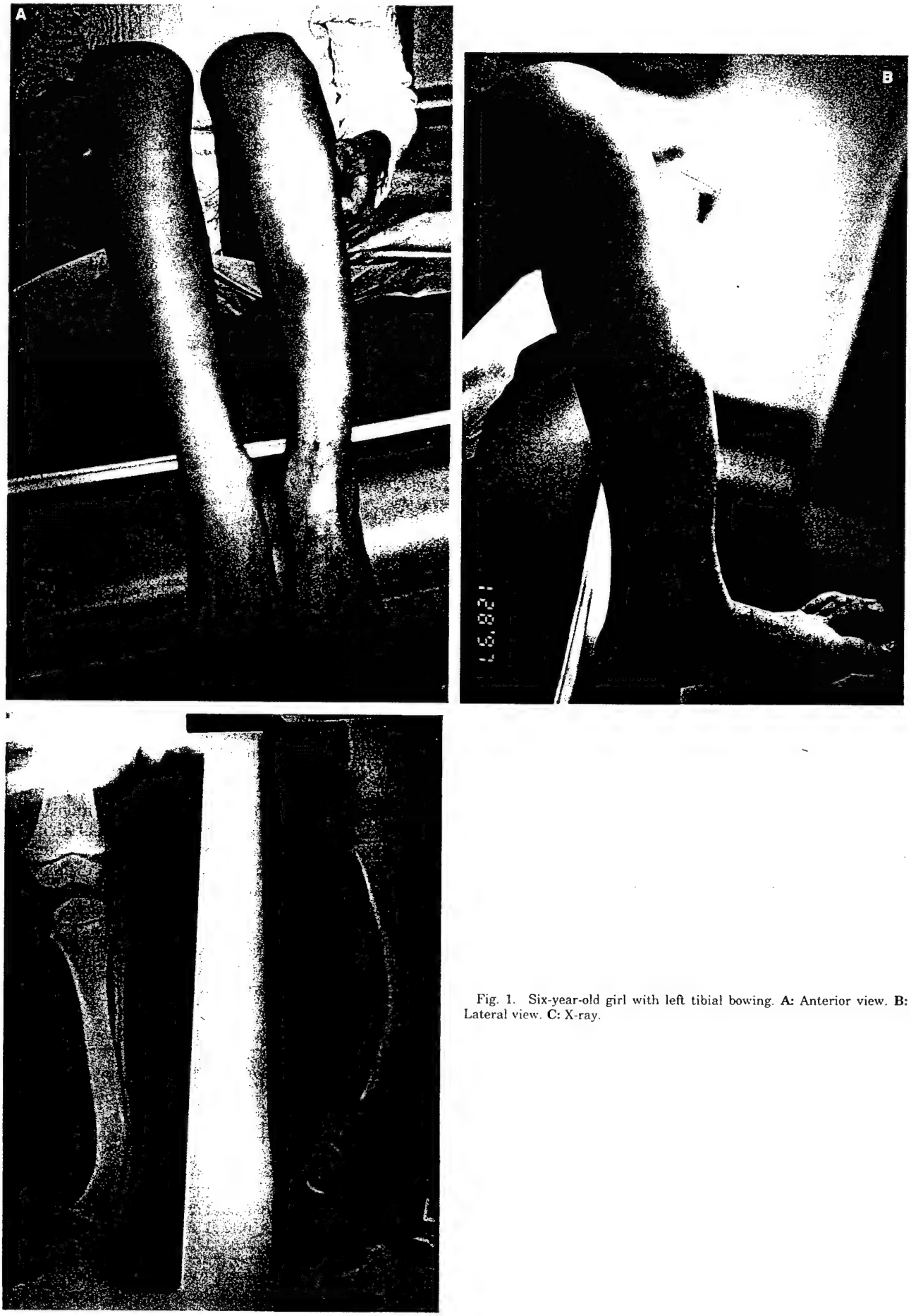

Fig. 1. Six-year-old girl with left tibial bowing. **A:** Anterior view. **B:** Lateral view. **C:** X-ray.

Cases with complications of fracture, pseudarthrosis, surgery, and/or amputation secondary to the bowing were placed in Group 2.

Selection of Control Individuals

Control subjects were only used in Study A. Initially each of the affected individuals from the database was age- and clinic-matched to two control subjects (NF1 patients without bowing/pseudarthrosis). Control subjects were age-matched to pseudarthrosis-affected individuals within 1 year in all cases under 40 years of age. Cases over 40 years of age were matched to within 4 years of control subjects (the oldest individual was 54 years old). Evaluation of the affected patients and their selected matched control subjects identified a few individuals who did not fulfill the NF1 diagnostic criteria. They were eliminated from the study leaving 172 NF1 controls and 85 NF1 individuals affected with pseudarthrosis.

Statistical Analysis

In Study A, NF1 patients with pseudarthrosis were compared to matched NF1 control subjects for gender, mode of inheritance, and associated manifestations. Analysis included Fisher exact tests, Mann-Whitney U tests, and chi-squared calculations using SYSTAT version 5.0.

RESULTS

Study A

Study A analyzed the frequency of 40 different manifestations of NF1 in the control and pseudarthrosis-affected groups. Some include café-au-lait macules, Lisch nodules, discrete neurofibromas, plexiform neurofibromas, optic gliomas, seizures, hydrocephalus, developmental abnormalities, heart disease, endocrine abnormalities, Noonan phenotype, other minor anomalies, and asymmetry unrelated to pseudarthrosis or bowing. Table I summarizes results from a representative sample of some of the more common findings in NF1. When the patients with long bone dysplasia were compared to the NF1 control subjects there was no statistically significant difference in the frequency of any of the 40 features. Likewise, there was no significant difference between trait frequency in Group 1 and Group 2.

Information on whether or not the patient had an affected parent was available in the database on only 53% of cases and 34% of control subjects. Information on maternal versus paternal inheritance was available on all of these familial cases. In Study A, nine people inherited NF1 from their mothers and 12 from their fathers. In the control group, the numbers were 21 and 16 respectively. In this small group there is no statistically significant parent-of-origin effect ($P = 0.41$; Table II).

Most patients were Caucasian in both the control group (82.2%) and the pseudarthrosis-affected group (81.2%); 0.6% of controls and 5.9% of pseudarthrosis-affected cases were of African descent and 6.9% of controls and 9.4% of pseudarthrosis-affected cases were of Asian descent.

There was an excess of affected males (54 males to 31 females). This differed significantly from the control group, in which there were 85 males and 87 females ($\chi^2 = 4.0$, $P = 0.046$, using a two-tailed test with Yates' correction; Fig. 2). Almost the entire difference comes from the male predominance in Group 2 (36 males to 16 females). Group 1 had 18 affected males and 15 affected females.

Study B

The natural history of NF1 patients with long bone dysplasia was addressed through data from a questionnaire. Often the health care provider either did not completely fill out the questionnaire or indicated that the information was not available. For this reason, denominators are not consistent in all categories. Study B included 75 patients. The average age of cases was 11.9 years (N = 72; range = 0.5–54 years, median = 8.6 years). In 53 of the 75 cases the age of bone deformity recognition by a health care provider was established. The mean age of presentation of the osseous problem was 15.3 months (N = 18; range = 0–108 months, median = 8.5 months) in Group 1 and 25.7 months (N = 35; range = 0–228 months, median = 8 months) in Group 2. The combined age of presentation of all cases was 22.2 months (N = 53; range = 0–228 months, median = 8 months). In 36 of 53 patients, in whom the age of recognition of the bone deformity was recognized, the abnormality was identified before one year of age (Table III).

Females averaged fewer operations than males (Table IV). One patient underwent 13 operations while others achieved union with simple casting. In Group 2,

TABLE I. Study A: Common Clinical Manifestations of NF1 With and Without Pseudarthrosis (From NNFFID in Vancouver, BC)

Clinical manifestation	Prevalence in Group 1 (N)	Prevalence in Group 2 (N)	Prevalence in control group (N)	*P*-Value[a]
≥6 Café-au-lait macules	0.79 (33)	0.88 (52)	0.88 (172)	$P = 0.35$
Intertriginous freckling	0.48 (33)	0.37 (52)	0.29 (172)	$P = 0.08$
Scoliosis	0.18 (33)	0.33 (52)	0.23 (172)	$P = 0.23$
Dysplastic vertebrae	0.09 (33)	0.02 (52)	0.08 (172)	$P = 0.27$
Dysplastic sphenoid wing	0.00 (11)	0.00 (11)	0.07 (45)	$P = 0.55$
Plexiform neurofibroma	0.21 (33)	0.19 (52)	0.24 (172)	$P = 0.71$
Lisch nodules	0.21 (33)	0.35 (52)	0.36 (172)	$P = 0.25$
Glioma	0.03 (33)	0.06 (52)	0.12 (172)	$P = 0.18$
Seizures	0.00 (33)	0.06 (52)	0.05 (172)	$P = 0.40$

[a]2 × 3 Chi-squared analysis looking at differences in distribution across three categories: bowing, complicated, and controls.

TABLE II. Parent of Origin (Study A: Database Component)*

	Familial		
	Maternal	Paternal	De novo
NF1 control (N = 59)	21	6	22
Total affected (N = 45)	9	12	24
Group 1 (N = 18)	2	7	9
Group 2 (N = 27)	7	5	15

*No information available on 153 individuals (cases plus control subjects). Familial vs. de novo: (Group 1/Group 2): Fisher exact test (two-tailed) $P = 0.77$; (Control/total affected): Fisher exact test (two-tailed) $P = 0.12$. Maternal vs. Paternal: Fisher exact test $P = 0.41$.

TABLE III. Age Bony Deformity Recognized (Study B: Questionnaire Component)

	No. of cases	
Age (years)	Group 1	Group 2
At birth	3	7
0–1	10	15
1–2	3	4
2–3	1	3
>3	1	6
Total (N = 532)	18	35

16% (8/50) had an amputation. The average number of operations in Group 2 was 2.9 (range 0–13).

The average age of fracture in pseudarthrosis-affected cases was 4.61 years (N = 32) with a range of 0–28 years with females fracturing an average of 1.06 years later than males (Table IV). In Group 2, 53% fractured before the age of 2 years (Table V).

It was noted that 43% of cases had fibular dysplasia. Two patients had fibular dysplasia without tibial dysplasia. The spectrum of fibular dysplasia ranged from simple bowing to frank pseudarthrosis. Three patients had forearm deformities. They consisted of left ulnar pseudarthrosis with left radial bowing, isolated right radial pseudarthrosis, and right radial and ulnar bowing without pseudarthrosis. The remainder of cases had unilateral tibial and/or fibular deformities. Data analysis was restricted to long bone dysplasia of the tibia and fibula excluding ulnar and radius pseudarthroses.

Laterality of the affected bone was evenly distributed with 35 patients presenting on the left side and 34 patients presenting on the right side. All subjects presented unilaterally. Only three patients out of 71 had a neurofibroma near the site of the deformity.

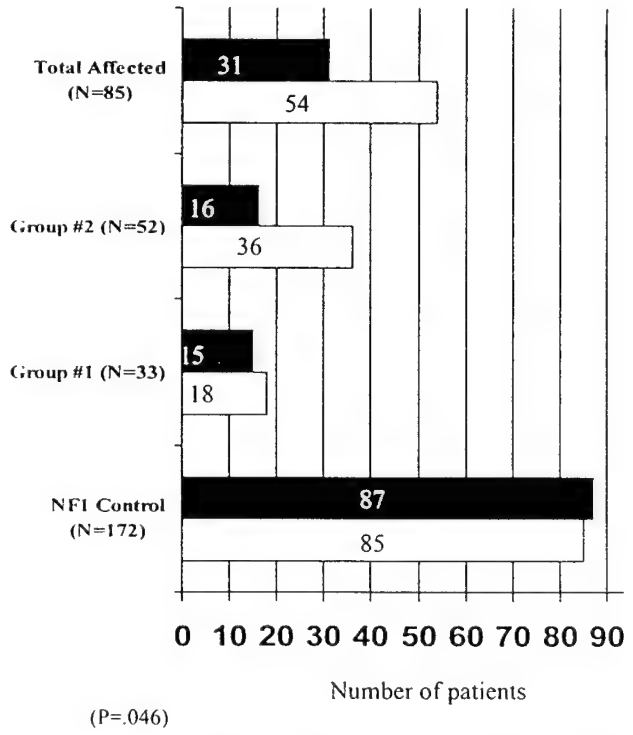

Fig. 2. Study A (database component) gender distribution (females, solid bars; males, open bars).

DISCUSSION

It is known that patients with NF1 exhibit a wide variety of manifestations. This study examined a number of variables with respect to pseudarthrosis (Table I). In Study A, there was no significant difference in the frequency of other clinical manifestations between pseudarthrosis-affected individuals and the control NF1 group. Morrissy et al. [1981] reported an increased observation of gliomas of the central nervous system among individuals with NF1 and pseudarthrosis. This study did not find an association of optic gliomas or neoplasms of any kind among patients.

We identified certain aspects of the natural history of pseudarthrosis in NF1, which may help practitioners more appropriately advise their patients on the potential complications. There are controversies concerning treatment of bowing/pseudarthrosis, and various investigators report different approaches to therapy. Some report one surgical procedure to be better than other procedures [Boyd and Sage, 1958; Charnley, 1956; Farmer, 1952; McFarland, 1951; Moore, 1949; Morrissy et al., 1981; Paterson et al., 1980; Wilson, 1941]. Some claim that amputation should not be done [Sofield, 1971; Van Nes, 1966] while others recommend amputation [Aitken, 1959; Boyd and Fox, 1948; Rathgeb et al., 1974; Rudicel, 1987]. Some patients seek amputation for therapy [Andersen, 1976b; Morrissy, 1981; Van Nes, 1966] due to the many complications that leave them incapacitated and disabled. Orthopedic surgeons remain frustrated on how best to handle this difficult condition. There has been much discussion on the various surgical procedures available to the orthopedist for management of long bone pseudarthrosis. With respect to bowing, the prevention of a fracture seems paramount. Some patients elected to have the bowed bone operated on before a fracture occurs, making it difficult to readily assess when or if a fracture would occur. Several surgical procedures including bone grafting, osteotomy, and vascularized autogenous grafts have been performed. Chronic bracing with a knee-ankle-foot orthosis (KAFO) is advocated by some orthopedists as a modality to prevent bowing from progressing to frank pseudarthrosis [Crawford, 1986]. At the present time there is no standardized protocol or

TABLE IV. Gender Observation of Fracture Age and Operations (Study B: Questionnaire Component)*

	Male	Female	Total	
Average age at fracture (years)	4.4	5.4	4.6	(Range = 0–28 yrs)
	(N = 24)	(N = 8)	(N = 32)	
	(Median = 2.0)	(Median = 2.5)	(Median = 2.0)	
Average no. of operations	3.3	1.8	2.9	(Range = 0–13)
	(N = 28)	(N = 10)	(N = 38)	
	(Median = 2.5)	(Median = 2.0)	(Median = 2.0)	

*Mann-Whitney U test: age at fracture: $P = 0.67$; number of operations: $P = 0.15$.

controlled clinical trial that has rigorously shown to be of value.

This study showed that half of the cases that fractured did so before the age of 2 years. However, the age was highly variable, ranging from prenatal to 28 years. Fifty-nine percent of Group 1 (patients with simple anterolateral bowing) were over the age of 4.61 years (our calculated average age of first fracture) with the oldest being 15.3 years old. This leaves nine patients with bowing who had not reached the average age at which fractures occurred.

It is commonly thought that NF1 patients with tibial bowing will inevitably sustain a fracture. Since this is a retrospective cross-sectional investigation, we were unable to determine from our data if these patients will fracture, but our data suggests that there are patients with significant bowing who may never fracture.

Fibular dysplasia often occurred with tibial dysplasia. In Study B, 43% of cases had fibular dysplasia. Two patients had fibular dysplasia without tibial dysplasia. Long bone dysplasia in NF1 is commonly referred to as tibial pseudarthrosis, but the fibula is often concurrently involved. Pseudarthroses of other long bones besides the tibia and fibula have been reported in NF1 patients. Other affected bones include the ulna, radius, humerus, femur, and clavicle [Rudicel, 1987]. Only a few isolated cases of these pseudarthroses have been reported.

We observed three patients with forearm deformities. They consisted of a case with left ulnar pseudarthrosis and left radial bowing, a case with right radial pseudarthrosis, and a case with right radial and ulnar bowing. None of these three cases had involvement of the tibia.

All cases presented with unilateral deformities. Laterality of the affected bone was evenly distributed in Study B. This observation suggests that other factors play a role in the development of this skeletal dysplasia, and that the NF1 mutant allele is not sufficient to cause the osseous dysplasia. These other factors could likely be somatic mutations of modifier genes.

The pathophysiology of pseudarthrosis is unknown. It has been postulated that a neurofibroma at the site of the deformity actually causes the deformity. Green and Rudo [1943] reported a histological specimen with a neurofibroma growing in the pseudarthrosis segment. Another study by Aegeter [1950] claimed that the tissue surrounding the site of the pseudarthrosis was the cause of the bony deformity. Brooks and Lehman [1924] proposed that the neurofibromas may arise from the nerves of the periosteum, erode into the bone, and then become covered by a shell of bone. However, Crawford and Bagamery [1986] stated that few surgical specimens have neurofibromatous tissue at the pseudarthrosis site and Moore [1941] found no report of actual invasion of the shaft by the neurofibroma. Our study confirms the latter observations. Only three patients of 71 had a neurofibroma near the site of the deformity. These may be incidental or part of the intrinsic dysplasia. Our data do not support the notion of a neurofibroma causing this osseous dysplasia.

There has been some speculation about a parent-of-origin effect. Miller and Hall [1978] noted an increased occurrence of serious complications such as pseudarthrosis when NF1 had been inherited from the mother as opposed to the father. These serious complications included pseudarthrosis. From their study it was postulated that there may be a parent-of-origin effect in the development of pseudarthrosis in patients with NF1. Study A did not suggest a maternal influence, but the numbers are small. There may be a maternal influence in other severe manifestations of NF1; however, these data do not support a parent-of-origin effect in the occurrence of pseudarthrosis (Table II).

Regarding racial distribution, it has been noted that few black Americans have NF1-associated optic nerve gliomas [Saal et al., 1995]. Therefore, there may be a correlation between ethnic origin and various NF1 manifestations. Due to the small number of non-Caucasians (17.8% of controls and 18.8% of affected individuals were non-Caucasian or "unknown"), no statistically significant conclusions could be made regarding racial distribution in this study. It is important to evaluate a larger number of patients of African and Asian descent to determine if the prevalence of pseudarthrosis in NF1 individuals from different ethnic backgrounds varies.

The most striking and singular observation of this study was the gender difference. In Study A, the control group showed an equal distribution of males and

TABLE V. Age at Fracture in Group 2 (Study B: Questionnaire Component)*

Age (years)	No. of cases
At birth	2
0–1	9
1–2	6
2–3	4
3–6	3
6–13	5
>13	3

*(N = 32); range, 0–28 years; mean, 4.6 years.

females, which is consistent with the literature on NF1 patients. In contrast, we found a significant excess of males with long bone dysplasia, especially in Group 2. The natural history study showed that male patients in Group 2 averaged more surgeries with an earlier age of fracture than females (Table IV).

This series is the largest investigation of patients with pseudarthrosis and NF1 reported to date. Previous studies do not provide information on gender, or include fewer patients. Although Gilbert and Brockman [1995] reported that males had a longer healing time than females, and Moore [1957] reported a slight male predominance in pseudarthrosis, neither study differentiated pseudarthrosis patients with NF1 and those without. We found no evidence in the literature to refute our findings.

The observation of a male predominance in the complicated group suggests that male gender could be a susceptibility factor for pseudarthrosis in patients with tibial bowing and NF1. This observation is also noted in NF1-associated leukemias. An increased proportion of males has also been observed among NF1 patients with myelogenous dysplasia [Shannon et al., 1992]. Conceivably, the mesodermal derivation of the tissue of origin of bone marrow cells and skeleton plays a role in the male susceptibility.

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Use of the NIH Criteria for Diagnosis of NF1 in Children

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ABSTRACT

Objective: The NIH Diagnostic Criteria for Neurofibromatosis 1 (NF1) are very useful clinically, but some individuals who are later shown to have NF1 cannot be diagnosed in early childhood using these criteria. The aim of this study is to determine the value of the NIH Diagnostic Criteria for NF1 in early childhood, to determine the age at which diagnosis can confidently be made, and to clarify the age at onset of the cardinal clinical features used in the NIH Diagnostic Criteria.

Methods: We studied 1893 NF1 patients from the National Neurofibromatosis Foundation International Database to determine the age at which the features included in the NIH Diagnostic Criteria appear.

Results: About 46% of sporadic NF1 cases fail to meet the NIH Diagnostic Criteria by 1 year of age. Almost all (98%; 0.95 CI = 96-100%) NF1 patients meet the criteria for diagnosis by age 8, and all do so by age 20. The usual order of appearance of the clinical features listed as NIH criteria is café au lait macules, axillary freckling, Lisch nodules, and neurofibromas. Symptomatic optic glioma is usually diagnosed by age 3 years, and characteristic osseous lesions are usually apparent within the first year of life.

Conclusion: The diagnosis of NF1 cannot always be made in young children using the NIH Diagnostic Criteria. Modification of these criteria may be necessary for children under 8 years of age.

Key Words

Neurofibromatosis 1, NF1, NIH Diagnostic Criteria, Natural History

INTRODUCTION

Neurofibromatosis 1 (NF1) is a progressive multisystem disorder. This autosomal dominant disease affects between 1/2000 and 1/4500 people[1, 2]. Half of all NF1 cases are familial, and half are caused by new mutations[2-5]. NF1 is diagnosed using a set of clinical criteria developed by a National Institutes of Health Consensus Conference in 1988[6] and recently reaffirmed by another group of experts[7]. To meet the NIH Diagnostic Criteria for NF1, an individual must demonstrate at least 2 of the 7 features listed in Table 1.

Table 1. The National Institute of Health Diagnostic Criteria for Neurofibromatosis 1

These criteria are thought to be very reliable in distinguishing adults with and without NF1 on the basis routine clinical and ophthalmological examinations[7, 8]. The reliability of these criteria for diagnosing NF1 in children has not been rigorously assessed, but some NF1 patients clearly cannot be diagnosed in early childhood by the NIH Diagnostic Criteria[9, 10].

Earlier diagnosis of NF1 may be beneficial to both affected children and their families. Genetic counseling could be offered to parents and other relatives earlier, and interventions for learning or developmental problems could be initiated sooner[11]. Diagnosis of NF1 may also be important in order to implement supportive care and to allow earlier detection of serious complications.

We used clinical information from the National Neurofibromatosis Foundation International Database (NNFFID) [12] to assess the value of the NIH Diagnostic Criteria in children.

METHODS

Three groups of patients were selected from the NNFFID for study. The first group consisted of 1402 patients, including both probands and affected relatives, under the age of 21 who were diagnosed as having NF1 by the NIH Diagnostic Criteria on their first visit to a participating NF Clinic. Patients who had not received an ophthalmological examination with a slit lamp were excluded. For purposes of classifying café-au-lait spots, we defined "prepubertal" as less than 13 years old and "postpubertal" as age 13 years or older. The number of cardinal clinical features that contribute to the NIH Criteria present in each patient was determined and plotted against age in three-year intervals.

A second study group consisted of 39 NF1 patients from the NNFF International Database who did not meet the NIH Criteria for diagnosis on their first recorded examination but who did so on a subsequent examination. Only patients who were re-examined at periodic intervals of 4 years or less were included in this group.

Finally, we analyzed the individual cardinal clinical features in each of 1893 NF1 patients who were diagnosed as having NF1 on their first examination at a participating NF Clinic to determine age-specific prevalence rates for each feature. This group of patients includes the 1402 patients in the first group as well as patients who met the NIH criteria for NF1 but have not had a slit lamp examination. The total number of patients included for each cardinal clinical feature varies because we excluded cases in which the presence or absence of the particular feature could not be determined unequivocally from available

data.

95% confidence intervals were calculated for all data using the method for proportions described in Zar, 1984 [13]. This method uses the relationship between an F distribution and a binomial distribution to compute a confidence interval for the binomial parameter.

RESULTS

The frequencies by age of 2, 3, 4 or 5 or more of the clinical features that comprise the NIH Diagnostic Criteria among 1402 patients with NF1 are shown in Figure 1. The patients in this analysis include both sporadic cases and those who have inherited an *NF1* mutation from an affected parent. Some familial cases are included even if they only exhibit one cardinal clinical feature because the NIH Diagnostic Criteria permit a diagnosis of NF1 for patients who have one such feature and a positive family history.

Almost all of these NF1 patients (97%; 0.95 CI = 94%-98%) have two or more of the cardinal clinical features included in the NIH Diagnostic Criteria by age 8. All of the NF1 patients have 2 or more cardinal clinical features by age 20. In contrast, 30% of NF1 patients under the age of 1 year have only one of the cardinal clinical features. These infants must all have an affected first degree relative in order to be diagnosed with NF1.

Figure 1. Age-specific frequency of 2,3,4, or 5 or more clinical features included in the NIH Diagnostic Criteria among 1402 NF1 patients under the age of 21.

All of the patients included in Figure 1 were diagnosed with NF1 on their first visit to a participating NF Clinic. We also studied 39 NF1 patients with an average age of 1.6 years who did not meet the NIH Diagnostic Criteria on first examination but were diagnosed as having NF1 by these criteria at a later date. The ages of these patients when

they were found to meet the NIH Diagnostic Criteria are shown in Figure 2. These patients were found to have at least 2 of the cardinal clinical features at an average age of 4.6 years, with a maximum age of 19. These findings support our observation in Figure 1 that almost all NF1 patients meet the NIH Diagnostic Criteria by the age of 8 years, and all patients do so by age 20.

Figure 2 - Age at which 39 NF1 patients meet 2 or more criteria

The progressive nature of NF1 results in an increased frequency of many of the cardinal clinical features with age. In order to determine the usual sequence of appearance of the cardinal clinical features, we plotted the prevalence of each feature among 1893 NF1 patients (probands and affected relatives) under the age of 21 years. The ages at which these patients met the NIH criterion for café-au-lait macules is given in Figure 3, for neurofibromas in Figure 4, for inguinal or axillary freckling in Figure 5, for Lisch nodules in Figure 6, for optic glioma in Figure 7, and for distinctive osseous lesions in Figure 8.

Ninety-nine percent of NF1 patients have 6 or more café-au-lait macules greater than 5 mm in diameter by 1 year of age. Inguinal or axillary freckling affects a maximum of 90% of NF1 patients by 7 years old. Lisch nodules affect more than 70% of patients by age 10. Neurofibromas are present in 48% of 10-year-old patients and 84% of 20-year-old patients. Symptomatic optic glioma is diagnosed in the first year of life in 1% of NF1 patients and reaches maximum frequency (about 4%) by the age of 3 years.

Characteristic osseous lesions are usually apparent within the first year of life and occur in about 14% of NF1 patients.

Figure 3 - Cafe-au-lait macules - Age at which 1888 NF1 patients have cafe au lait spots

Figure 4 – Inguinal or Axillary Freckling - Age at which 1835 NF1 patients have inguinal or axillary freckling

Figure 5 - neurofibromas – Age at which 1891 NF1 patients have two or more neurofibromas of any type or one plexiform neurofibroma

Figure 6 - Lisch nodules - Age at which 1402 NF1 patients have two or more Lisch nodules

Figure 7 - Optic Glioma - Age at which 1807 NF1 patients have symptomatic optic glioma

Figure 8 - Osseous Lesions - Age at which 1722 NF1 patients have a distinctive osseous lesion

DISCUSSION

The number of cardinal clinical features included in the NIH Diagnostic Criteria increases in NF1 patients with age. As shown in Figure 1, 70% of NF1 patients can be diagnosed as having the disease before they are one year old on the basis of 2 or more of these cardinal clinical features. The other 30% of NF1 patients under the age of 1 year have only one of the cardinal clinical features. These children must all have an affected first degree relative in order to be diagnosed with NF1 according to the NIH Criteria. We would expect an equal number of sporadic cases to have only one cardinal feature by 1 year of age because about half of all NF1 patients represent sporadic cases[2-4]. These sporadic cases would not be diagnosed as having NF1 before 1 year of age because they do not meet the NIH Diagnostic Criteria.

If the total number of NF1 patients diagnosed by one year of age is N, the number of sporadic cases that remain undiagnosed by age one is $0.3\,N$. The total number of individuals with *NF1* mutations at one year of age is $(N + 0.3\,N)$, half of whom represent sporadic cases. The proportion of sporadic *NF1* cases that remain undiagnosed by one year of age is, therefore

$$\frac{0.3N}{1/2(N+0.3N)} = 46\%$$

From this we estimate that about 46% of sporadic cases lack 2 or more of the cardinal clinical features and cannot be diagnosed by the NIH Diagnostic Criteria by one year of age. NF1 probably cannot be diagnosed using these criteria in at least 5% of the sporadic cases by age 8. All NF1 probands have at least two of the cardinal clinical features

included in the NIH Diagnostic Criteria by 20 years of age.

The age-specific prevalence rates of the individual cardinal clinical features that comprise the NIH Diagnostic Criteria are displayed in Figures 3 to 8. The usual order of appearance of these features is café au lait macules, axillary freckling, Lisch nodules, and neurofibromas. Optic glioma is usually diagnosed within the first three years of life, and characteristic osseous lesions within the first year.

Café-au-lait macules, which are present within the first year of life in most NF1 patients, are not likely to develop after the age of 4 if not already present. Inguinal or axillary freckling is commonly present by the age of 7 years and does not usually develop after this age. Lisch nodules usually develop before the age of 10 years. Neurofibromas are usually present by the age of 20 if they are going to develop.

The frequencies of the individual cardinal clinical features seen in this study are similar to those previously reported [1, 3, 14, 15]. The data used for these analyses are cross-sectional in nature and are, therefore, not ideal for examining progression of features. A more accurate representation of NF1 patients could be obtained from a large longitudinal study, but only limited longitudinal data are available.

The diagnosis of NF1 cannot always be made or ruled out with confidence in young children using the NIH Diagnostic Criteria. Diagnosis of NF1 can confidently be made using these criteria by age 8 in most children and by age 20 in all of them. However, we

recommend that young children who have 6 or more café au lait spots greater than 5mm in largest diameter but do not meet any of the other cardinal clinical features be followed as if they had NF1. Almost all of these children will develop other clinical manifestations of NF1 with time [9,10].

Modification of the NIH Diagnostic Criteria may be necessary to provide more reliable diagnosis for young children. The inclusion of other clinical features, which are both sensitive and specific for NF1in pediatric patients, may improve diagnosis. Short stature [16], macropcephaly [16] and unidentified bright objects on head magnetic resonance imaging [17,18,19] have been suggested as potentially useful in this regard. Routine radiographic screening for common osseous lesions, such as pseudarthrosis, sphenoid wing dysplasia, and dysplastic vertebrae, should also be considered. We are currently studying the value of adding these features to the NIH Criteria.

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Table 1. National Institute of Health Diagnostic Criteria for Neurofibromatosis Type 1.

Cardinal Clinical Features (Any two or more are required for diagnosis)[1]
Six or more café au lait macules over 5mm in greatest diameter in prepubertal individuals and over 15 mm in greatest diameter in postpubertal individuals.
Two or more neurofibromas of any type, or one plexiform neurofibroma
Freckling in the axillary or inguinal regions
Optic glioma
Two or more Lisch nodules (iris harmartomas)
A distinctive osseous lesion such as sphenoid dysplasia or thinning of the long bone cortex with or without pseudarthrosis.
A first degree relative (parent, sibling, or offspring) with NF1 by the above criteria

Figure 1. Age-specific frequency of 2, 3, 4, or 5 or more clinical features included in the NIH Diagnostic Criteria among 1402 NF1 patients under the age of 21 (including 95% confidence intervals).

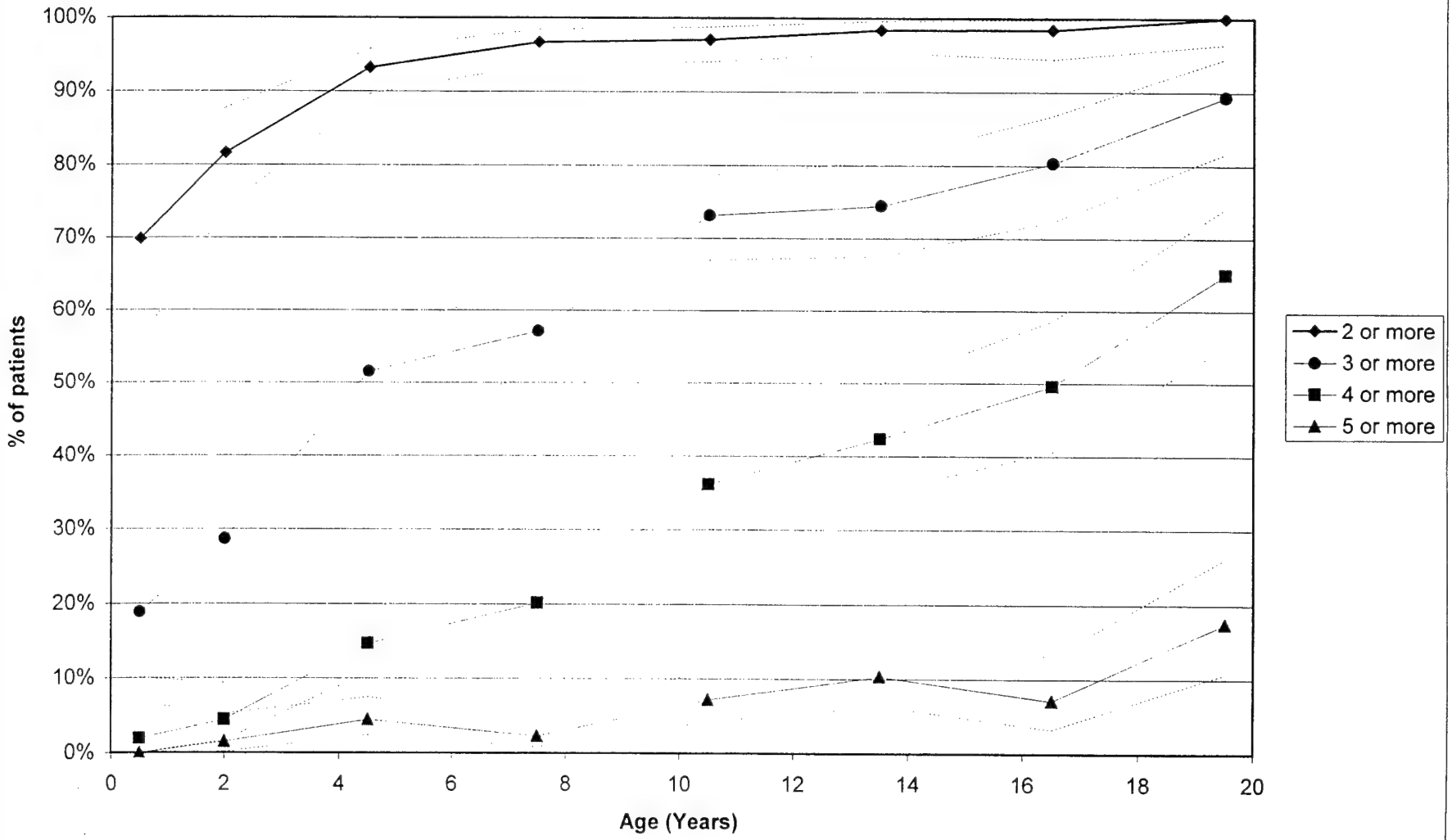


Figure 2. Age at which 39 NF1 patients meet 2 or more NIH diagnostic criteria

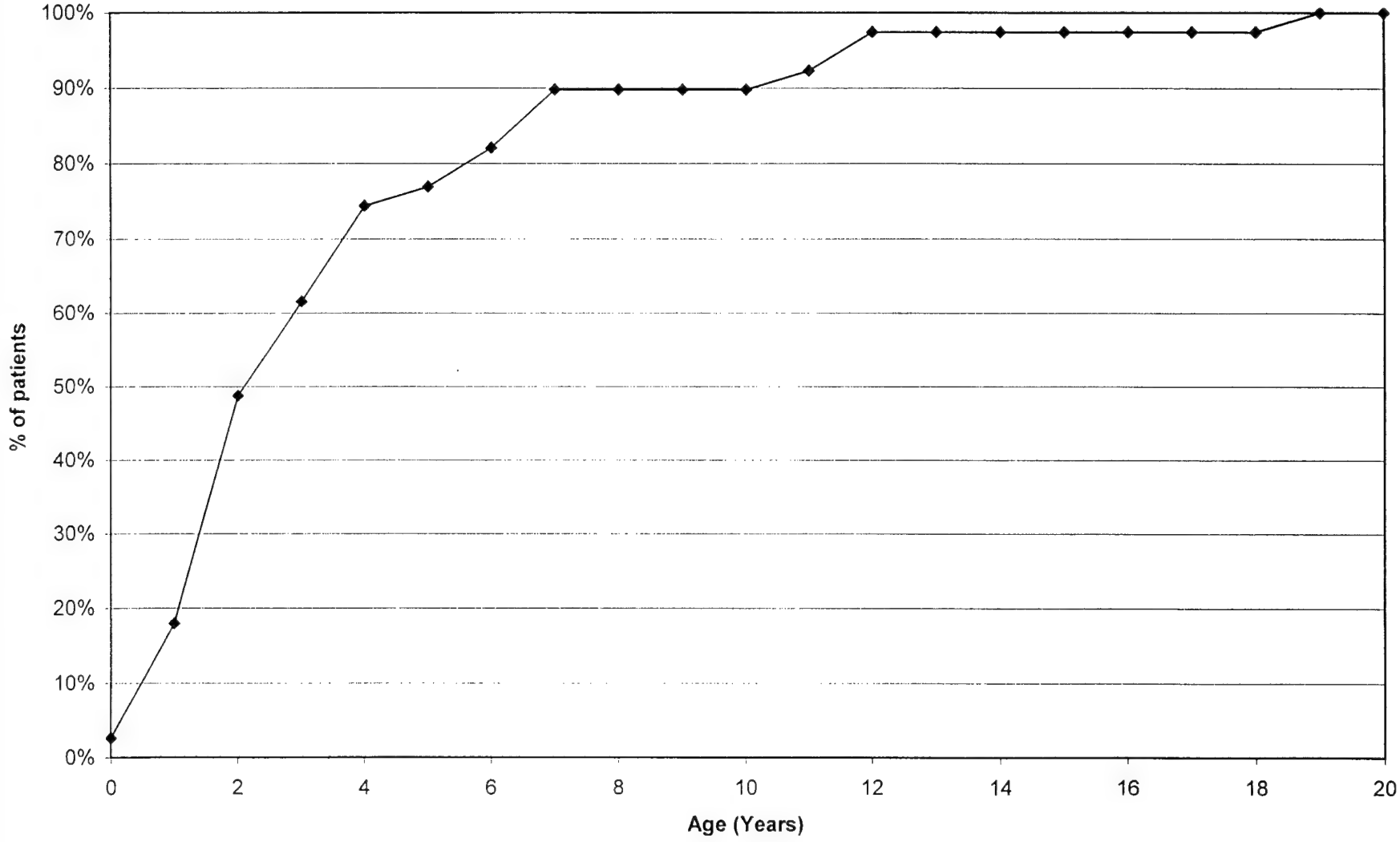


Figure 3. Age at which 1888 NF1 patients have cafe au lait spots

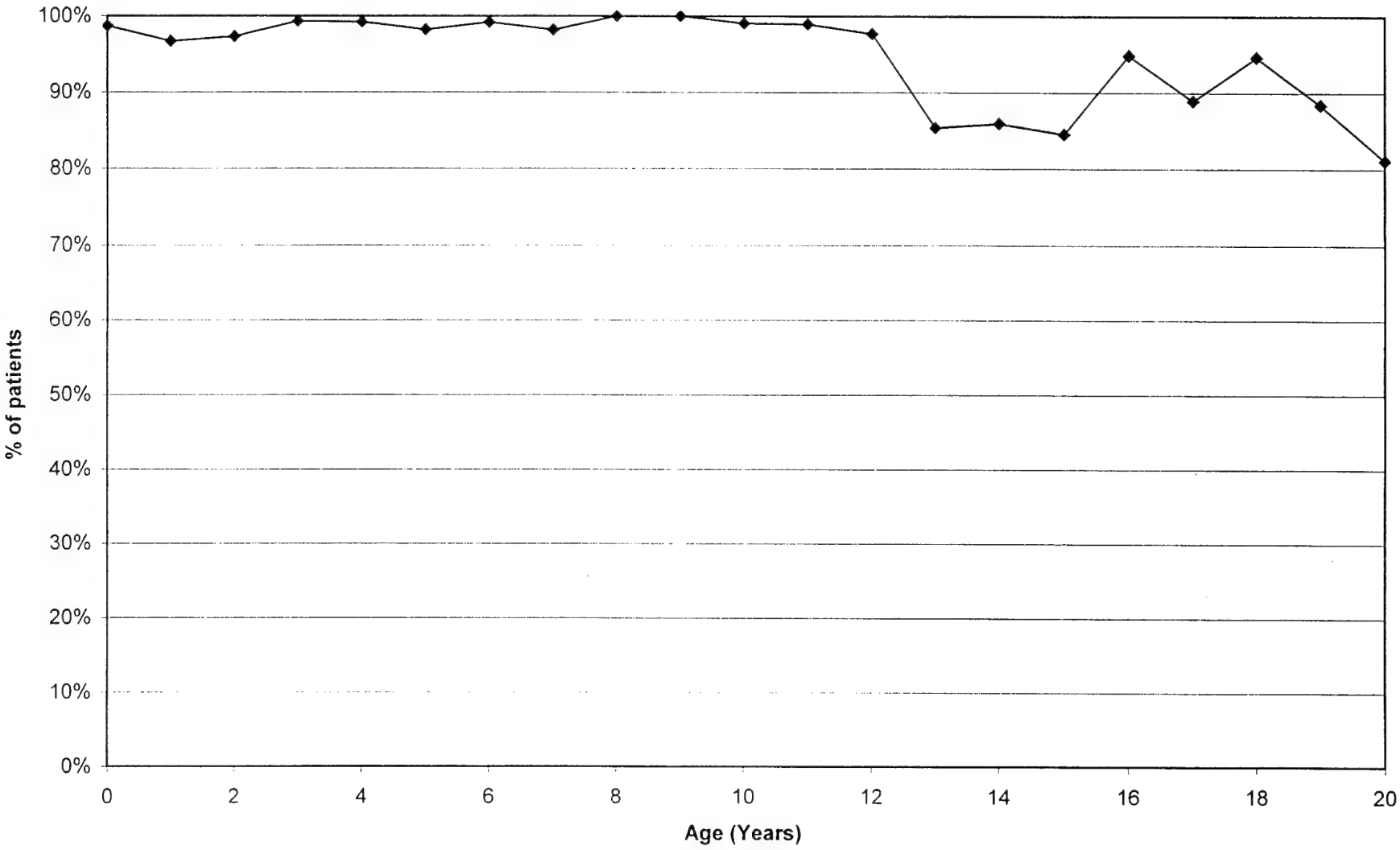


Figure 4. Age at which 1835 NF1 patients have inguinal or axillary freckling

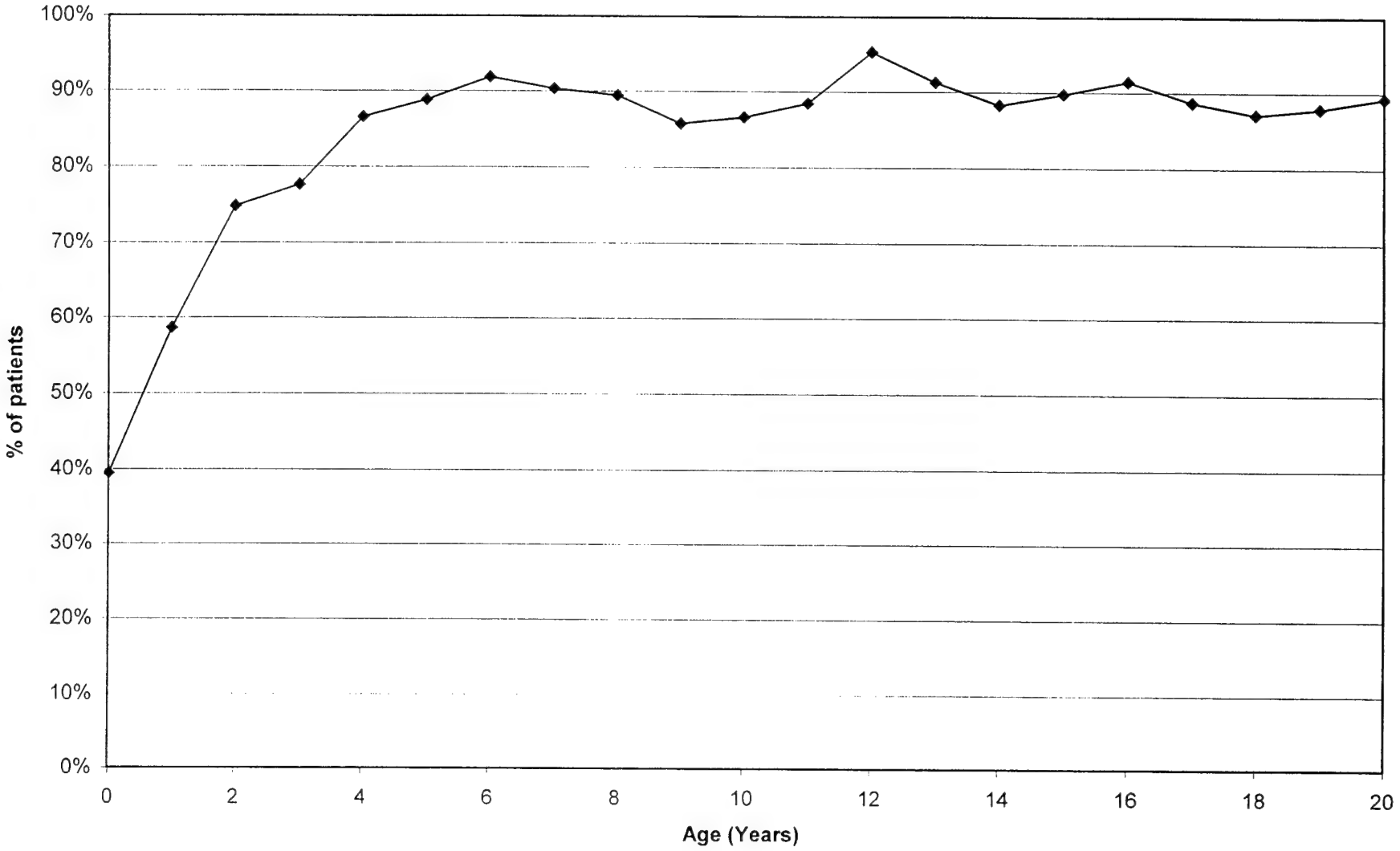


Figure 5. Age at which 1891 NF1 patients have two or more neurofibromas of any type or one plexiform neurofibroma

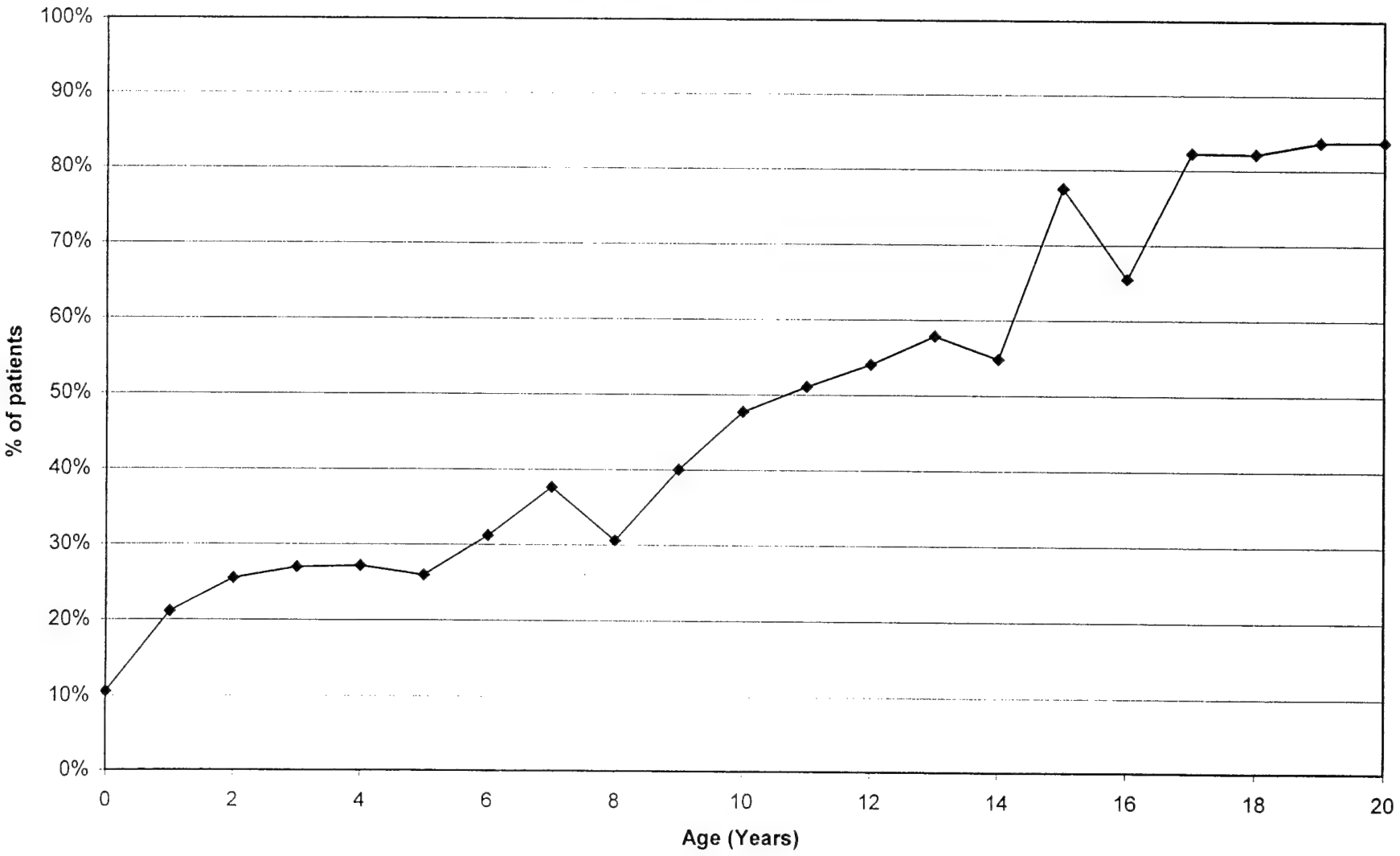


Figure 6. Age at which 1402 NF1 patients have two or more Lisch nodules

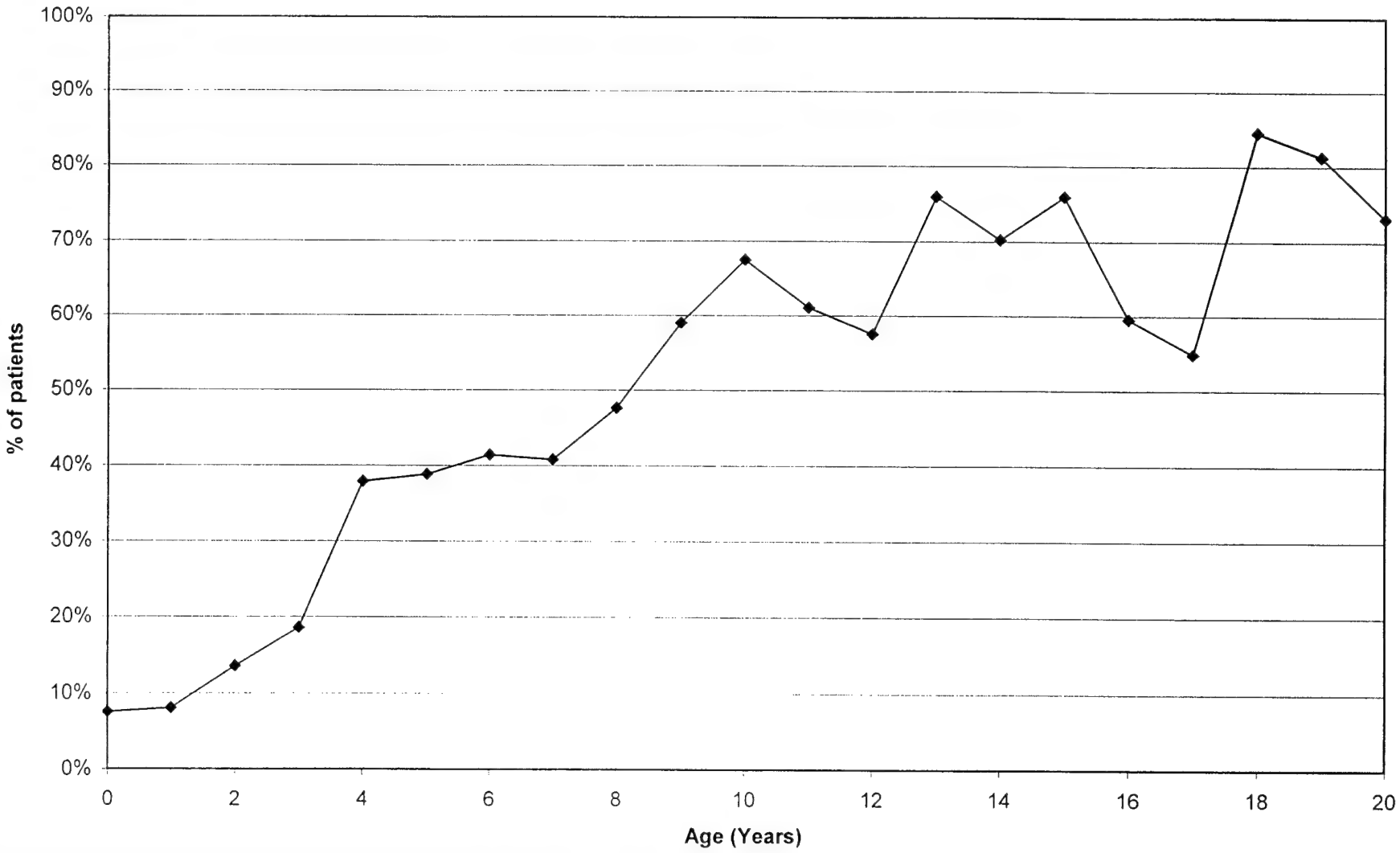


Figure 7. Age at which 1807 NF1 patients have symptomatic optic glioma

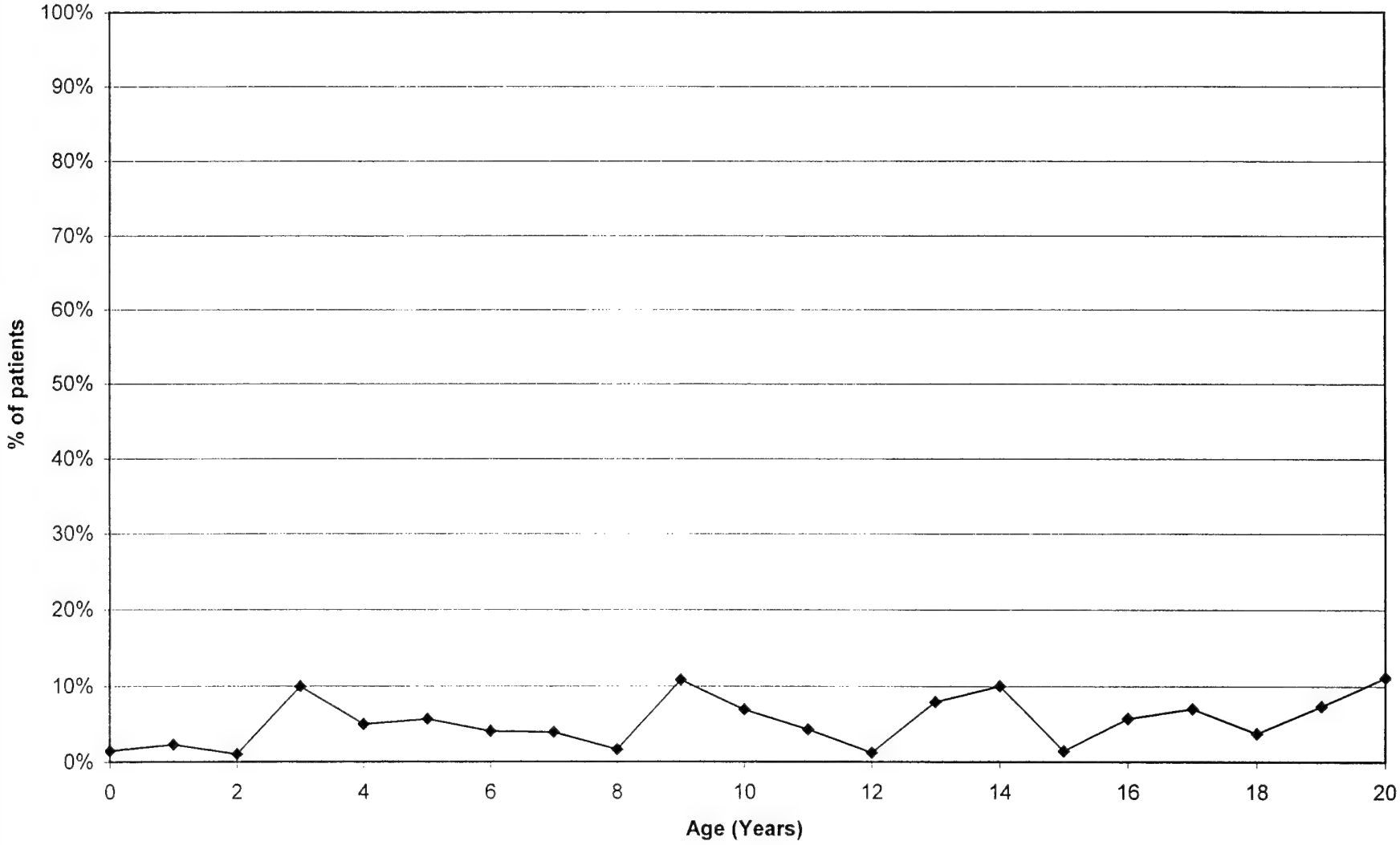
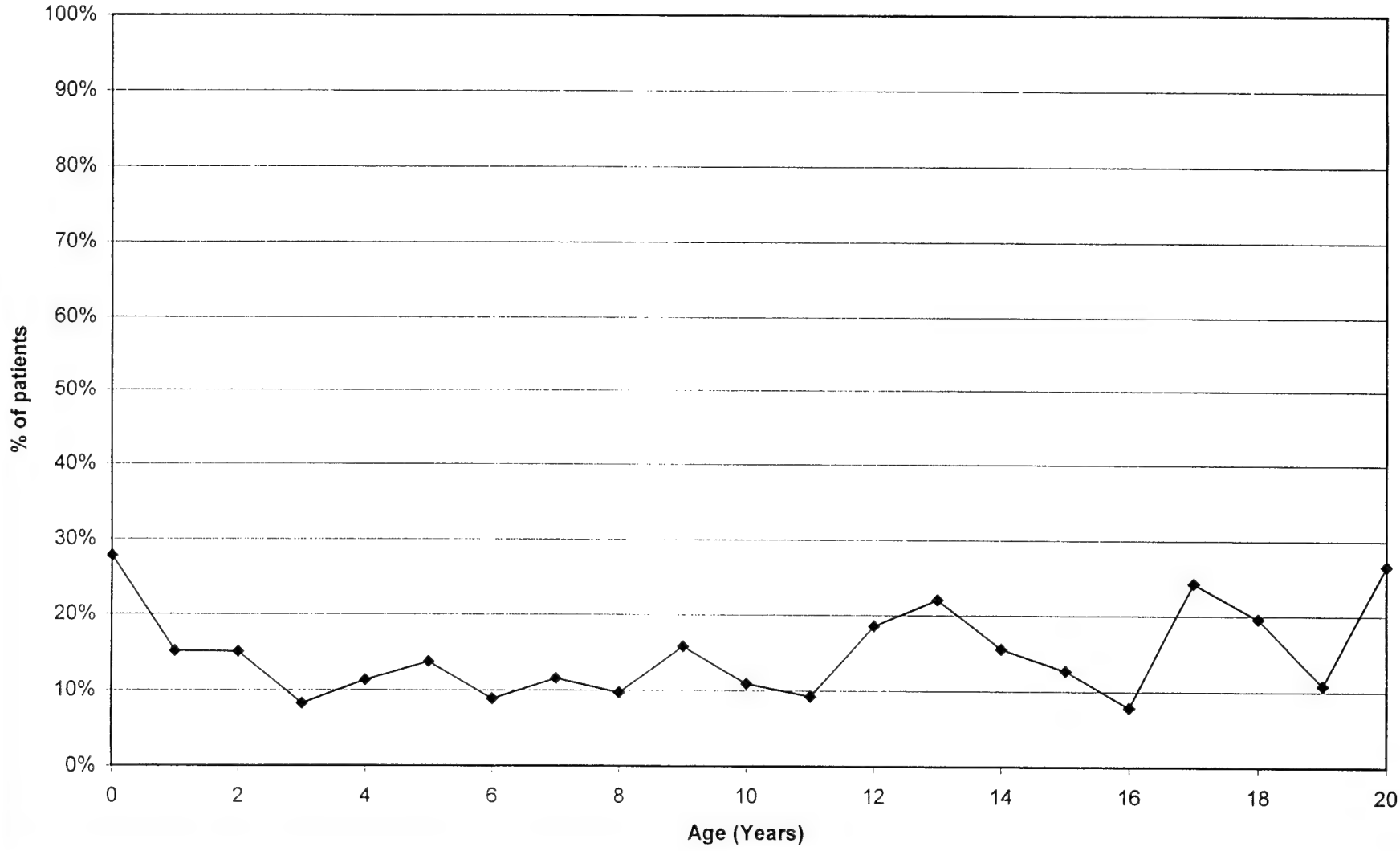


Figure 8. Age at which 1722 NF1 patients have a distinctive osseous lesion



The Use of Unidentified Bright Objects on MRI for Diagnosis of Neurofibromatosis 1 in Children.

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Unidentified Bright Objects (UBOs) have been observed on cranial magnetic resonance imaging (MRI) in 45-80% of children with neurofibromatosis 1 (NF1). Because the NIH Diagnostic Criteria often do not permit unequivocal identification of NF1 in young children, UBOs have been proposed as an additional diagnostic criterion. We examined the sensitivity and specificity of UBOs for NF1 in 19 affected children and 19 age-matched controls. We measured the agreement of UBO recognition between two experienced pediatric neuroradiologists who independently examined the MRI studies on these patients. We also used data from 858 NF1 patients between the age of 2 and 21 from the NFDB (NNFF International Database) who had cranial MRI scans to determine whether the addition of UBOs to the existing NIH diagnostic criteria would improve early diagnosis.

The overall sensitivity of UBOs for NF1 averaged 97%, and the specificity averaged 79%. Agreement between the two radiologists was 84% overall (95% C.I. 67-100%). We do not routinely obtain cranial MRI examinations on NF1 patients, so our subjects as well as our controls are more likely to have central nervous system pathology than unselected children. Adding UBOs as a diagnostic criterion would permit 85% rather than 80% of two-year old NF1 patients from the NFDB to be diagnosed, a difference that is not significant. UBOs occur in children who do not have NF1, but the frequency and clinical significance in normal children need to be defined. UBOs may be more reliable for diagnosis of NF1 if they can be better characterized and differentiated from similar lesions that occur in other patients. Our data suggest that requiring a patient to have three or more UBOs in the cerebellum, medulla, pons, midbrain, thalami or lentiform nuclei is a more specific criterion for NF1 than the simple presence of such lesions.

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USE OF "UNIDENTIFIED BRIGHT OBJECTS" ON MRI FOR DIAGNOSIS OF NEUROFIBROMATOSIS 1 IN CHILDREN

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KEY WORDS

Neurofibromatosis 1, Unidentified Bright Objects, Diagnosis, Magnetic Resonance Imaging

ABSTRACT

Background: "Unidentified Bright Objects" (UBOs) have been observed on magnetic resonance imaging in 45-80% of children with neurofibromatosis 1 (NF1). Because of this high frequency and the fact that the NIH Diagnostic Criteria often do not permit diagnosis of NF1 in an affected individual during early childhood, UBOs have been proposed as an additional diagnostic criterion for NF1.

Methods: We examined the specificity and sensitivity of UBOs for NF1 in 19 affected children and 19 age-matched controls. We also measured the agreement of recognition of UBOs between two experienced pediatric neuroradiologists who independently examined the MRI studies on these patients.

Results: The sensitivity of UBOs for NF1 averaged 98%. The specificity averaged 79%. Agreement between the two radiologists was 84% overall (95% confidence interval 67-100%). Using data from the National Neurofibromatosis International Database, we estimate that adding UBOs as a diagnostic criterion would permit 76% rather than 67% of two-year old NF1 patients to be diagnosed. The value of including UBOs is less in older patients because a larger proportion of them meet the existing diagnostic criteria.

Conclusion: The presence of UBOs is not a useful diagnostic criterion for NF1. UBOs may be more reliable for diagnosis of NF1 if they can be better characterized and differentiated from similar lesions that occur in other patients.

INTRODUCTION

Neurofibromatosis 1 (NF1) is diagnosed using a set of clinical criteria that were developed by a National Institutes of Health (NIH) Consensus Conference in 1988 [1] and recently reaffirmed by another group of experts [2]. These criteria are thought to be very reliable in distinguishing adults with and without NF1 on the basis of routine clinical and ophthalmological examinations [2,3]. However, most of the clinical features upon which the diagnosis is based are uncommon in infants with NF1 and increase in frequency with age [4-6]. As a consequence, more than 40% of 2-year old children with sporadic NF1 cannot be diagnosed using the NIH Criteria [4].

The NIH Diagnostic Criteria were developed before magnetic resonance imaging (MRI) was commonly used as a screening technique [2] in patients with NF1. Many features of NF1, including optic gliomas, orbital neurofibromas, other gliomas, and "Unidentified Bright Objects" (UBOs) are apparent on cranial MRI examination [7]. UBOs are areas of increased T2-weighted signal intensity that cannot be visualized on T1-weighted imaging or CT scan [8]. They show no mass effect or contrast enhancement [9]. Pathologically, they correspond to areas of vacuolar or spongiotic change in the brain substance [10-13]. UBOs are found in 45-80% of pediatric NF1 patients [2,9-11,20-23]. UBOs evolve over time and are more common in children than adults with NF1 [9,17-21]. No consistent relationship has been found between the learning disabilities that frequently occur in NF1 patients and the presence of UBOs, although a correlation between the location of these lesions and IQ score has been suggested [14-16]. Because they are so common in pediatric patients with NF1 [19,24], UBOs have been proposed as a diagnostic criterion in young patients who are suspected to have NF1 [22,25,26]. Earlier diagnosis of

NF1 would be beneficial to both affected children and their families. Genetic counseling could be offered to parents and other relatives earlier, and interventions for learning or developmental problems could be initiated sooner [27]. On the other hand, MRI studies are expensive and require anesthesia in small children. The use of UBOs in the diagnosis of NF1 has also been criticized because of the lack of information on the specificity of UBOs to NF1 patients [2]. Although UBOs are thought to be uncommon in children who do not have NF1 [22], the frequency in normal children is unknown. Lesions that resemble UBOs have occasionally been reported in adults who do not have NF1 [28,29].

We examined the specificity and sensitivity of UBOs for NF1 and measured the agreement between two radiologists who independently examined the MRI studies on a series of NF1 patients and age-matched controls. We also assessed the effect of adding UBOs to the NIH Diagnostic Criteria on our ability to diagnose NF1 in young patients. We found that UBOs are not a useful diagnostic criterion for NF1.

METHODS

Two age-matched groups of children between the ages of 4 and 10 years seen for MRI scanning at British Columbia's Children's Hospital in Vancouver, Canada, were selected. Twenty NF1 patients from the Vancouver clinic who underwent routine cranial MRI scanning were identified through the National Neurofibromatosis Foundation International Database (NNFFID) [30]. One of these patients was excluded from the study because he has optic gliomas, which would permit a radiologist to diagnose probable NF1 and might, therefore, bias the recognition of UBOs. The control group consisted of 19 children without NF1 who immediately followed each of the NF1 patients in the radiology logbook, had a head MRI scan for other reasons, and whose age matched within one year of the corresponding NF1 patient. Six of the 19 control patients had undergone radiation therapy for tumours, six had seizures, two had developmental delay, three had metabolic abnormalities, one had malformations, and one had post-infectious demyelination.

We used axial fast spin echo weighted images, 3555/16/80/21/192x256/ 5mm/1mm/2 (TE/TE/FOV/Matrix/Thickness/Gap/Average). These 38 images were examined independently by two experienced pediatric neuroradiologists. The studies were read in a random order, and the radiologists were unaware of the age or NF1 status of the patients. The presence or absence of UBOs and their locations were recorded for each patient. The sensitivity and specificity of UBOs for NF1 were calculated using standard formulae[31]. Concordance between the radiologists' interpretations was calculated using the kappa statistic[32].

A second group of NF1 patients, including both probands and affected relatives between the ages of 2 and 21 years who were diagnosed with NF1 by the NIH Diagnostic Criteria on their first visit to any participating NF Clinic, was identified through the NNFFID. Patients who had not received an ophthalmological examination with a slit lamp to look for Lisch nodules were excluded. 858 patients who had cranial MRI examinations were selected from this group, and the presence or absence of UBOs in each patient was determined. The frequency of UBOs was plotted against age in one-year intervals. We also plotted the Baysian-corrected frequency of patients by age who met 2 or more of the current NIH Diagnostic Criteria (i.e., the proportion of patients in whom NF1 can be diagnosed), and the Bayesian-corrected proportion who would meet 2 or more criteria if UBOs were added as an additional criterion. 95% confidence intervals of the frequency were calculated by the method of Zar[33].

RESULTS

Cranial MRI examinations on 19 NF1 patients and 19 age-matched controls who did not have NF1 were reviewed blindly by two experienced pediatric neuroradiologists. The NF1 patients had a mean age of 7.9 years. Ten were male and 9 were female. The control patients had a mean age of 7.8 years. Eleven were male and 8 were female. Radiologist #1 identified UBOs in 18 (95%) of the 19 NF1 patients and in 3 (16%) of the 19 control subjects. Radiologist #2 identified UBOs in all 19 NF1 patients and in 5 (26%) of 19 control subjects. The five control subjects in whom Radiologist #2 identified UBOs included the three in whom Radiologist #1 identified UBOs.

The sensitivity of UBOs identified by Radiologist #1 for NF1 in this study was 95%. The specificity was 84%. The sensitivity of UBOs identified by Radiologist #2 for NF1 was 100%, and the specificity was 74%.

Kappa statistics were calculated to estimate the agreement in identification of UBOs between the two radiologists. The overall agreement was 84% (95% confidence interval 67-100%). Table 1 shows the frequency of identification of UBOs in various anatomic regions of the brain on MRI scans of the children with NF1 and their age-matched controls. Agreement in identification of UBOs between the two radiologists varied from 29% to 79% in various regions of the brain. As shown in Table 1,UBOs occur most frequently in the cerebellum, medulla, pons, midbrain, thalami, and lentiform nuclei.

Figure 1 shows the frequency of UBOs by age among 870 NF1 patients from the NNFFIDB who had cranial MRI scans. The frequency of UBOs did not vary greatly with

age among these patients, all of whom were between 2 and 21 years old. UBOs were diagnosed in about 45% of these pediatric patients with NF1.

Figure 2 shows the proportion of these patients who met two of the standard NIH Criteria and thus can be diagnosed with NF1 at various ages. The figure also shows the effect of adding UBOs as a diagnostic criterion. The proportion of patients who meet 2 or more diagnostic criteria is not affected substantially by the inclusion of UBOs as an additional diagnostic criterion. At the age of 2 years, 67% of NF1 patients can be diagnosed using the current NIH Diagnostic Criteria. 76% have two or more of the features necessary to permit diagnosis if UBOs are added as a diagnostic criterion. Due to the progressive nature of NF1, the value of including UBOs is less in older patients because a larger proportion of them meet the existing diagnostic criteria.

DISCUSSION

UBOs were identified by MRI examination in all or almost all of the 19 NF1 patients studied between the ages of 4 and 10 years and were also identified in 16%-26% of the age-matched children who do not have NF1. This relatively high frequency of UBOs among children without NF1 was unexpected, but the fact that UBOs were identified independently in 3 of these 19 subjects by both radiologists indicates that UBOs may often occur in children without NF1 who undergo MRI examination. . We do not routinely obtain cranial MRI examinations on NF1 patients, so our subjects are more likely to have central nervous system pathology than an unselected group. Similarly, the control group does not accurately represent the general population because MRI scans are only performed on children suspected of having central nervous system pathology. Eleven of the 19 control patients had disorders associated with white matter changes that might look like UBOs on MRI scan. These eleven control patients included six who had received radiation therapy for a tumour, three who had metabolic disorders, one with a malformation and 1 with post-infectious demyelination. Four of the five non-NF1 patients in whom UBOs were found had such central nervous system pathology.

The overall agreement in identification of UBOs between these two experienced pediatric neuroradiologists was 84% as estimated by the kappa statistic. We observed more differences in identification of UBOs in specific anatomic regions, with agreement as low as 49% in the internal capsule and 27% in the cerebrum (Table 1). This inconsistency between radiologists suggests that UBOs are often difficult to identify with certainty.

When the presence of UBOs was added to the NIH Diagnostic Criteria, the diagnosis of NF1 increased from 67% to 76% in affected 2 year-old children (Figure 2). Adding UBOs as a diagnostic criterion had little effect on the ability to diagnose NF1 in older children. In patients under two years of age, areas of increased intensity on T2-weighted images (UBOs) are difficult to see because incomplete myelination of the brain results in an increased T2-weighted signal from the grey matter [34-36]. Therefore, the presence of UBOs is unlikely to be a reliable diagnostic tool in children under 2 years of age.

UBOs may be more reliable for diagnosis of NF1 if they can be better characterized and differentiated from similar lesions that occur in other patients. UBOs were most commonly identified in the cerebellum, medulla, pons, midbrain, thalami, and lentiform nuclei of NF1 patients. Our data suggest that requiring a patient to have several UBOs in these areas may be a more specific criterion for NF1 than the simple presence of such lesions.

CONCLUSION

Despite the high sensitivity of UBOs for NF1, their presence is probably not a useful criterion for diagnosis of NF1 in young children. UBOs are difficult to identify with certainty because signal intensities are subjective and vary in children with various kinds of central nervous system pathology. UBOs are not specific for NF1; they may also be seen in other children undergoing cranial MRI examination. Further studies are necessary to determine if better characterization of the appearance, location, and number of UBOs provides more reliable criterion for diagnosis of NF1 in young children.

Table 1. Frequency and agreement in identification of UBOs in various anatomic regions of the brain on MRI scans of 19 children with NF1 and 19 age-matched control subjects.

Structure	Number of Subjects in Whom UBOs Were Identified				Kappa
	Radiologist #1		Radiologist #2		
	NF1 Patients	Controls	NF1 Patients	Controls	
Cerebellum	14	1	13	1	0.71
Brainstem (Total)	18	1	13	1	0.73
Medulla	14	1	1	0	
Pons	7	1	13	1	
Midbrain	0	1	13	1	
Basal Ganglia (Total)	16	0	18	2	0.79
Caudate	0	0	1	0	
Thalami	11	0	13	1	
Lentiform Nuclei	13	0	13	1	
Internal Capsule	3	0	8	0	0.49
Cerebrum (Total)	2	2	3	4	0.27
Frontal Lobe	0	0	1	1	
Temporal Lobe	0	1	0	0	
Parietal Lobe	2	2	3	3	
Occipital Lobe	0	1	0	0	

Legends for Figures

Figure 1. Frequency of UBOs by age among 858 NF1 patients from the National Neurofibromatosis Foundation International Database who had cranial MRI examinations. Dotted lines indicate 95% confidence intervals.

Figure 2. Age at which 858 NF1 patients between 2 and 21 years of age can be diagnosed by meeting 2 or more diagnostic criteria.

◆——— **Frequency using the current NIH Diagnostic Criteria [1,2]. Dotted lines indicate 95% confidence intervals.**

●——— **Frequency with UBOs as an additional criterion.**

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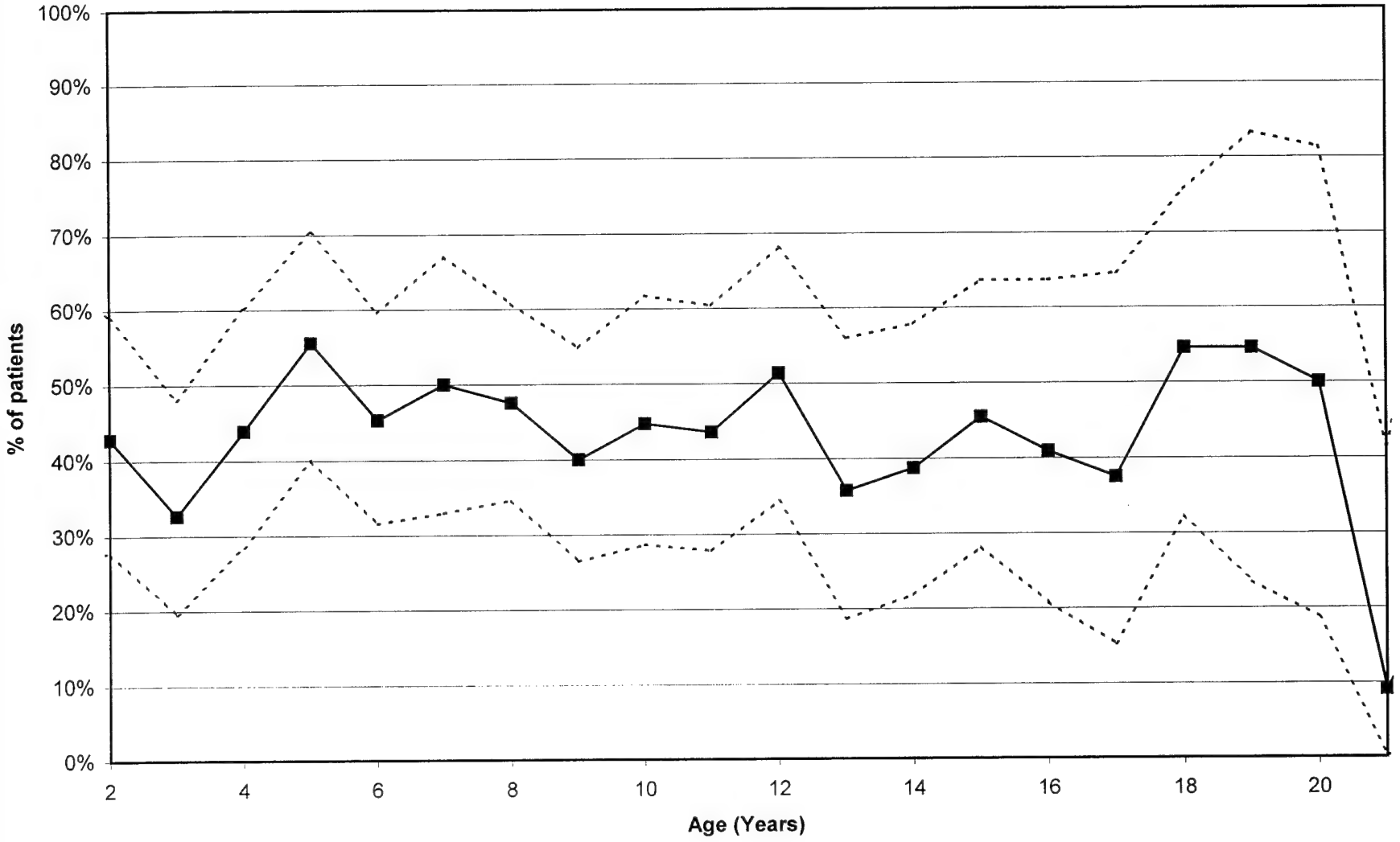
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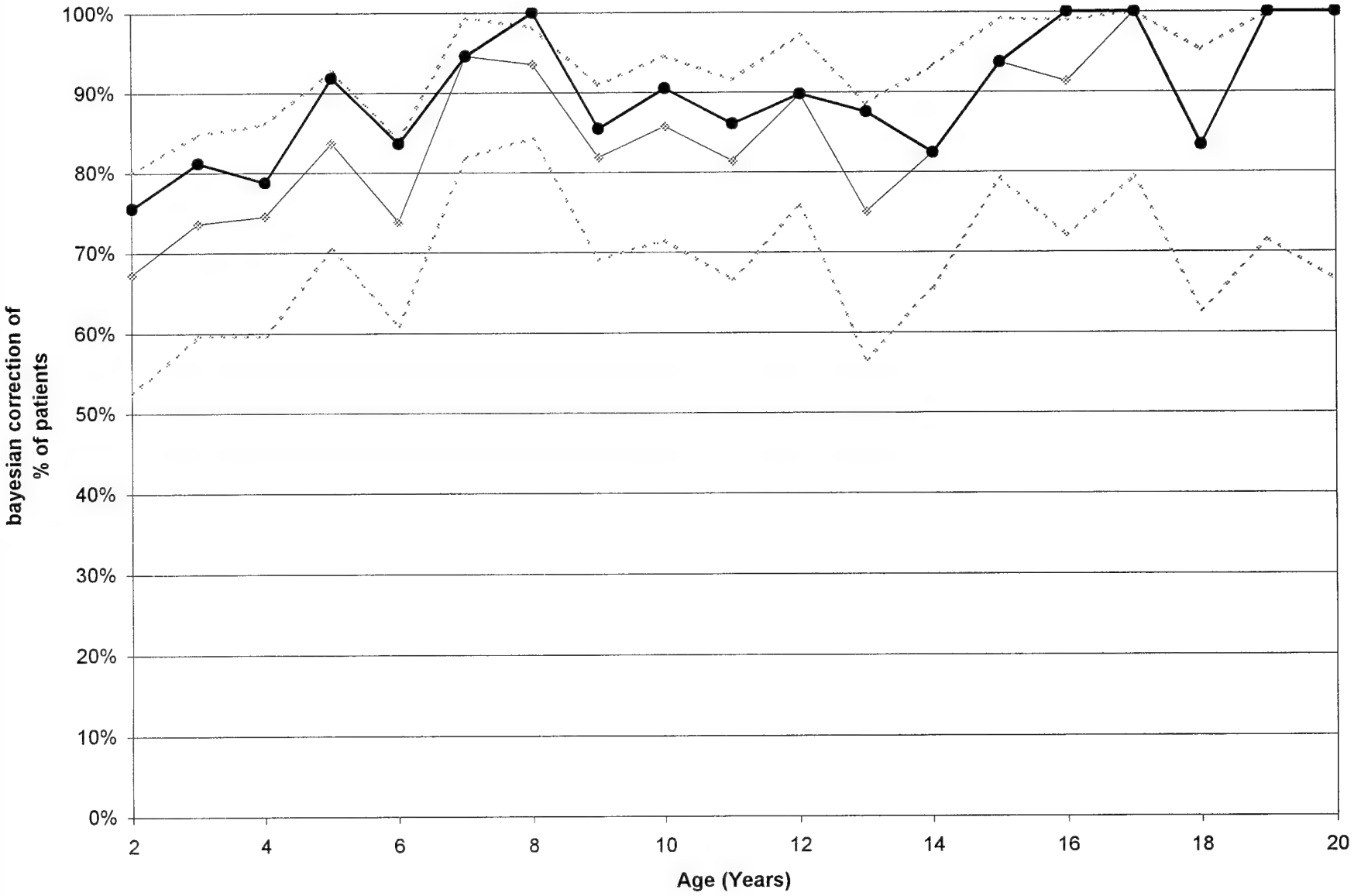
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Analysis of Growth in Neurofibromatosis 1 (NF1)

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ABSTRACT

Objective: To analyse the distributions of height, head circumference and body mass index in people affected with neurofibromatosis 1 (NF1)

Design: Cross-sectional database survey.

Setting: The National Neurofibromatosis Foundation International Database (NFDB) includes clinical information on NF1 patients from 25 participating centres in North America, Europe and Australia.

Subjects: 2538 Caucasian NF1 patients. 52% female and 48% male.

Main Outcome Measures: Height and head circumference measurements of NF1 patients were compared to age- and sex-matched population norms using z-score standardisation. Body mass index measurements were compared to norms using centile curves.

Results: The distributions of height and head circumference are shifted and *unimodal* among NF1 patients. 14% of patients have short stature. 20% have macrocephaly. 41% have macrocephaly when height is taken into account. Body mass index centiles are lower in NF1 patients than in population norms.

Conclusions: Alterations of height and head circumference are not limited to NF1 patients with frank short stature or macrocephaly. Macrocephaly is even more prevalent when height is taken into consideration. Obesity is less frequent among NF1 patients than in the unaffected population.

KEY WORDS

Neurofibromatosis 1, head circumference, height, weight, body mass index

INTRODUCTION

Neurofibromatosis 1 (NF1) is a generally progressive autosomal dominant disorder affecting about 1 in 3000 people [1-3]. Its most frequent features are café-au-lait macules, iris Lisch nodules, and discrete and plexiform (diffuse) neurofibromas. Short stature [4,5] and macrocephaly [4-7] are more common in people affected with NF1 than in the general population, while obesity may be less common [5 p182].

NF1 features such as scoliosis and early or delayed puberty occasionally influence height. However, short stature associated with NF1 usually affects the whole skeleton proportionately, and its cause is unknown [5 p153, 8]. Disease features such as hydrocephalus and plexiform neurofibromas occasionally affect measurements of head circumference. However, increased head circumference among NF1 patients usually has no obvious cause and appears to result from overgrowth of the brain [5 p 165, 8].

Riccardi has hypothesised that short stature [5 p153] and macrocephaly [5 p175] are "all-or-none" phenomena that affect only a portion of patients with NF1. According to this hypothesis, NF1 patients would be expected to fall into two distinct groups: those whose height falls into the same normal distribution as unaffected people of the same age and those whose height is decreased. NF1 patients would also be expected to fall into two distinct groups with respect to macrocephaly: those whose head circumference falls into the same normal distribution as unaffected people of the same age and those whose head circumference is increased.

We examined the distributions of these measurements to determine whether changes in growth affect the NF1 population as a whole or only a subset of patients. We

also examined the distribution of body mass index to test the clinical observation that obesity is uncommon among NF1 patients [5 p182].

SUBJECTS AND METHODS

Subjects

All patients included in this study meet the NIH Diagnostic Criteria for NF1 [9, 10]. Measurements of patient height, weight and head circumference were obtained from the National Neurofibromatosis Foundation International Database (NFDB) [11]. At the time of this analysis, the NFDB included extensive demographic and cross-sectional clinical information on 2538 Caucasian NF1 patients examined during 1980-98 at 25 participating centres in North America, Europe and Australia. Information was collected and recorded on each patient using a standard procedure. The data were routinely screened for quality and consistency by the database administrator. Only measurements from each patient's first visit to a participating clinic were included in the analysis. NF1 patients who were known to have any of the following features that may affect stature were excluded from analyses of height: pseudarthrosis [n=101(4%)], early (under age 10 years) [n=32(1%)] or delayed (over age 15 years) [n=51(2%)] puberty, optic glioma [n=215(8%)], scoliosis [n=533(21%)], vertebral dysplasia [n=81(3%)], or spinal compression [n=42(2%)]. Patients with any of the following features that may affect head size were excluded from analyses of head circumference: plexiform neurofibroma of the head [n=156(6%)], early or delayed puberty, optic glioma, or hydrocephalus [n=84(3%)]. Patients with early or delayed puberty were also excluded from analyses involving body mass index.

Measurements

We defined "height" as recumbent length in children less than 2.25 years of age and as stature in patients 2.25 years of age and over. Patients were measured without shoes. Short stature was defined as height 2 or more standard deviations below the reference population mean. "Head circumference" was defined as the fronto-occipital circumference and measured at the largest diameter over the occiput and forehead. Macrocephaly was defined as head circumference 2 or more standard deviations above the reference population mean. "Body mass index" (BMI) refers to weight in kilograms divided by the square of height in meters. Patients were weighed without shoes in light indoor clothes or examining gowns. Infants less than three months old were weighed with no clothes or only diapers.

Reference Populations

Standard population norms for height and body mass index by age were obtained from the National Center for Health Statistics (NCHS) studies [12,13]. Height measurements were taken during 1963-74, and body mass index during 1976-80. The NCHS standards are based on a sample consisting of 83 percent White or Hispanic subjects and 17 percent Black subjects living in the United States. Standard population norms for head circumference by age were obtained from the Fels Institute study conducted during 1929-75 [12]. The Fels Institute sample is slightly less heterogeneous than the NCHS sample. Norms for the head circumference-to-height ratio (head circumference in centimetres multiplied by 100 and divided by height in centimetres) were obtained from a Child Research Council longitudinal study conducted during 1927-67 and reported by McCammon [14]. The Child Research Council sample was of mixed

European stock, with the majority of northern European extraction. The majority of the subjects were at least second-generation residents of the United States.

Distribution Analysis

Head circumference and height measurements and the ratio head circumference/height were converted into z-scores above and below the standard means by the following formula:

$$z = \frac{\text{(measurement of patient)} - \text{(mean of the sex- and age- matched control group)}}{\text{standard deviation of the sex- and age-matched control group}}$$

This standardisation controls for both age and gender by categorising NF1 patients into the same age and gender groups as the population norms.

Single data entry has an error rate around 2% [15-17]. Patients with height and head circumference measurements corresponding to a z-score with an absolute value greater than 5 were excluded to minimise data entry errors. Twenty (1.2%) height and 7 (0.4%) head circumference measurements corresponded to z-scores below -5. Six (0.4%) height and 8 (0.4%) head circumference measurements corresponded to z-scores above 5. We expect that about 1% of the remaining measurements contain errors.

Distributions of z-scores for height, head circumference and head circumference/height were plotted in histograms using SAS [18]. Each histogram is based on the z-scores compiled from males and females of all ages. In addition, the modality of each distribution was quantified by computing its dip statistic [19]. Dip approaches zero

for unimodal distributions. The significance of a given dip value is determined by comparing it to the distribution of values from a known unimodal distribution.

Although each contributing centre was instructed to use the same procedures for measuring patients, variables such as operator and apparatus were not controlled. We, therefore, tested the standardised data by analysis of variance to determine if significant differences exist among the measurements made by the major contributing centres.

Growth Curves

The distribution of body mass index does not appear to be Gaussian in the standard NCHS controls of similar age [13]. Therefore, this measurement was not converted into a z-score. Instead, centiles were generated directly from the data for NF1 patients of various ages and compared to the corresponding centiles from reference populations. NF1 patients were divided into sex and age groups matching those of the curves available for population norms. A typical series of age-groups had medians of: 1, 3, 6, and 9 months and 1, 1.5, 2, 2.5, 3…18 years. Age-group limits were determined by splitting the difference between a given median and the next lowest and highest medians. Patients with ages equidistant from two medians were assigned to the older age group. For example, the 1.5 year-old group included patients ages 1.25 to 1.749 years old. The 5th, 25th, 50th, 75th and 95th centiles for body mass index were determined for each sex in each age group of NF1 patients and plotted along side the centiles from the corresponding population standards. The data were plotted and smoothed using SAS [18]. Smoothing was done by producing a cubic spline that minimises a linear combination of the sum of squares of the residuals of fit and the integral of the square of the second

derivative [18,20]. Smoothed curves were inspected to ensure that the final results reasonably represent the data. Splining was used and described in detail by Hamill et al. [12] to generate standard curves for the NCHS.

RESULTS

Analysis of variance for heterogeneity among major NFDB contributing centres revealed no difference ($p=0.64$) between age- and sex-standardised heights. There was significant heterogeneity ($p<0.01$) among major centres with respect to age- and sex-standardised head circumference measurements for these children; however, the mean z-score for each centre examined ranged between 0.6 and 0.7.

Figures 1, 2 and 3 show the distributions of standardised measurements of height, head circumference and head circumference/height among NF1 patients and population norms. Head circumference/height is used as a measure of relative head circumference. It addresses the possibility that NF1 patients may have large heads for their bodies.

Mean standardised height among NF1 patients is lower than mean height in the reference population. Fourteen percent of the NF1 patients lie two or more standard deviations below the reference population mean, compared to 2% of norms.

Mean standardised head circumference among NF1 patients is greater than mean head circumference in the reference population. Twenty percent of NF1 patients lie 2 or more standard deviations above the reference population mean.

Mean standardised head circumference/height ratio among NF1 patients is much greater than the mean value in the reference population. Forty-one percent of NF1 patients lie 2 or more standard deviations above the reference population mean.

The histograms for height, and head circumference/height appear unimodal (figs. 1-2). The modality of the histogram for head circumference (fig. 3) is not obvious. The dips of the distributions of height, head circumference and head/height ratio are 0.01444,

0.00965 and 0.00769, respectively. These values correspond to the 80th, 50th and 1st centiles, respectively, of dip values from known unimodal distributions. In other words, the dip values are either within the normal range of unimodal distributions or lower.

The skewness of the standardised height distribution is 0.11 ($p>0.20$), and the kurtosis is 0.92 ($p<0.002$). The standardised head circumference distribution has a skewness of –0.23 ($p<0.005$) and a kurtosis of 0.54 ($p<0.005$). The head circumference/height distribution has a skewness of 0.47 ($p<0.002$) and a kurtosis of 1.21 ($p<0.002$). Skewness will be zero when the distribution is a completely symmetric bell-shaped curve. Positive skewness indicates that the cases are clustered more to the left of the mean with most of the extreme values to the right. A negative value indicates clustering to the right. A normal distribution will have a kurtosis of zero. If the kurtosis is positive, then the distribution is more narrow than a normal distribution, while a negative value means it is flatter.

The distribution of BMI was examined without age- or sex-standardisation because the data are not normally distributed in the reference population [13]. Figures 4 and 5 show the centile curves for body mass index among males and females with NF1 between the ages of 2 and 40 years. The 5th and 50th centiles for BMI are quite similar for NF1 females and unaffected females at all ages. In striking contrast, the 95th centile for BMI is considerably lower among NF1 females than among unaffected females beginning in mid-childhood and continuing throughout life. The 5th, 50th and 95th centiles for NF1 males are all considerably lower than among unaffected males beginning in mid-childhood and continuing throughout life.

DISCUSSION

The NCHS and Fels standards were used for comparison to height and head circumference of NF1 patients because these studies cover a wide range of ages. The normal population studies were longitudinal and therefore more accurately represent growth than cross-sectional studies. Moreover, these standards are commonly used clinically to diagnose short stature and macrocephaly. Standards for head circumference/height are not included in either the published NCHS or Fels Institute studies and are rarely reported. The McCammon standards for height/head circumference are based on a larger and more representative sample than any other found in the literature.

We were concerned that differences between centres would increase the variability of our sample and diminish our ability to analyse the standardised distributions. However, analysis of variance detected no large differences for height and head circumference between the contributing centres.

Standardisation for age and sex by z-scores is a transformation that allows pooling of measurements across groups that may differ in age and sex. This transformation can be applied to measurements for which standard population distributions are approximately normal. Under these conditions, a subject's z-score for a particular measurement closely corresponds to his or her centile rank. The distributions of height, head circumference and head circumference/height satisfy this criterion [12,14]. The distribution of body mass index appears to deviate from normality after early childhood [13]. Therefore, standardized distributions were only calculated for height, head circumference and head

circumference/height. The distribution of body mass index was examined with centile curves, which involve no assumptions regarding the nature of the distribution.

The shifts in the standardised distributions of head circumference and height (figs. 1 and 2) confirm that, on average, NF1 patients are shorter and have bigger heads than standard populations. The shifts are very similar to those in a recent longitudinal study by Carmi et al. [21]. None of the norms were taken during the same years as the NFDB sample, and some were taken 20 or more years earlier. Secular trends suggest that height and head circumference may have increased in the normal population over this time [16, 22-24]. If year-specific standards were used instead in our study and the one by Carmi et al., the shift in stature may be slightly larger than indicated here and the shift in head circumference may be slightly smaller. Ascertainment bias may also affect the distribution shifts. Although short stature and macrocephaly are not among the diagnostic criteria for NF1 [9], these features may have contributed to the patients' referral to the contributing NF clinics. Therefore, the group in this study may be shorter and have bigger heads than the NF1 population as a whole.

The distributions of standardised height and head circumference are leptokurtic, indicating a relative excess of outliers. This variability could result from several factors. The patient measurements are cross-sectional and, therefore, more variable than longitudinal data. The patient group is geographically heterogeneous. As mentioned above, around one percent of the cases still contain data-entry errors. Ascertainment bias may increase the frequency of outliers. Alternatively, such distributions might represent composites of more than one normally distributed group with the same mean but different variances [25].

Riccardi has suggested that short stature [5 p153] and macrocephaly [5 p175] in NF1 are "all-or-none" phenomena, i.e., that two different groups of NF1 patients exist: those with short stature (or macrocephaly) and those without. Under this premise, the distributions should be bimodal, with one mode more than 2 standard deviations outside the normal mean. Our findings are not consistent with this suggestion. The dip values of head circumference, height and head circumference/height (figs. 1-3) indicate that height is reduced to some degree and head circumference enlarged to some extent in the NF1 patient population as a whole.

Body mass index (BMI) has a high correlation with body fat as estimated from body density, particularly when age is taken into consideration, and a low correlation with height. In the absence of skin-fold measurements, it is the best available index of obesity based on weight and height [26, 27]. 95^{th} centiles of body mass index (figs. 4 and 5) confirm the clinical observation that obesity is rare in people affected with NF1 [5 p.182]. The normal sample used for body mass index was measured 4-20 years before the NFDB sample. Body mass index may have increased in the normal population since that time [24] –obesity in NF1 patients may be even more infrequent by today's standards.

Patients with hydrocephalus and plexiform neurofibromas of the head were excluded from the analyses of head circumference, so enlargement of the head in the remaining patients must be due to enlargement of the scalp, skull or brain. In NF1, enlargement of the brain is the likely cause [5 p. 165, 8]. Glial cell proliferation is increased *in vitro* by sera from NF1 patients, compared to sera from unaffected individuals [28]. Optic or other CNS gliomas are another manifestation of glial cell proliferation. They were observed by MRI in 10% of people in the NFDB affected with

NF1. Other studies have observed optic gliomas in 1.5% of 135 and 15% of 217 NF1 patients [29,30]. Glial overgrowth is an important part of NF1 and it may be responsible for macrocephaly in NF1 patients.

Patients with puberty disturbance or bone abnormalities that can affect stature were excluded from analyses of height. The cause of the height reduction in the remaining NF1 patients is unknown, but it appears to affect the skeleton proportionately [5 p.153]. Data reviewed by Howell et al. [31] indicate that growth hormone replacement therapy resulted in a moderate increase in height for NF1 patients with biochemical evidence of growth hormone deficiency. However, growth hormone deficiency was found in only 3 (2.5%) of 122 children with NF1 in another study [32]. Short stature occurs much more frequently (15%) in the NFDB than can be attributed to such deficiency. Although growth hormone levels were not measured routinely in the NFDB patients, less than 1% are known to have ever had documented growth hormone deficiency.

The findings in this study are consistent with known molecular function of the *NF1* gene/protein. The *NF1* protein, neurofibromin, is involved in control of cellular growth and differentiation through the interaction of its GAP-related domain with p21ras and tubulin [33]. Neurofibromin is expressed in many different tissues, including the brain [34], and mutations in the GAP-related domain produce hyperactivity of p21ras, which leads to aberrant signalling for cell proliferation. This may contribute to increased glial (astrocyte) cell proliferation [34,35] and to enlargement of the brain in NF1 patients.

The *NF1* homologue in *Drosophila* acts as an activator of the cAMP pathway [36] as well as a negative regulator of Ras. *Drosophila* homozygous for either of two

particular *NF1* mutants that lack expression of NF1 protein are 20 to 25% smaller than flies of the parental strain [37]. This growth defect was rescued not only by an *NF1* transgene but also by expression of activated protein kinase A [37], suggesting protein kinase A functions downstream of or parallel to neurofibromin. Deficiencies in this pathway may contribute to a "smaller" phenotype in humans as well. Activated protein kinase A is known to stimulate proliferation in some cell types [38,39] and may normally contribute to body growth. This is consistent with the shorter stature and lower weight we have observed among people affected with NF1. It may also explain the rarity of obesity. Normal stimulation of the protein kinase A pathway also accelerates differentiation and inhibits proliferation of glial (oligodendrocyte) cells [40,41]. Neurofibromin involvement in or between [42] the protein kinase A and p21ras pathways may contribute to the larger heads observed in people diagnosed with NF1. However, the patients with the smallest stature did not also have the largest heads [results not shown]. There could be several reasons for this. Other functions of the *NF1* gene could be involved. Different NF1 mutations may have greater (or lesser) effect on height than on head circumference. Factors outside the NF1 gene could contribute to the height and head circumference disturbances in NF1.

Since both head circumference and height are affected in NF1, head circumference was also studied relative to height, using the ratio head circumference/height. The standardised distribution of head circumference/height (fig. 3) indicates that the heads of NF1 patients are even larger in comparison to population norms when height is considered. Carmi et al. [21] have reported this as well. About 40 percent of the patients from this sample have relative macrocephaly –i.e. a head

circumference/height ratio that is two or more standard deviations above the reference population mean. This ratio is elevated throughout life in people affected with NF1 [results not shown] and may, therefore, aid in the early diagnosis of NF1 in children who do not yet satisfy the NIH criteria.

Short stature and macrocephaly are well-recognised clinical features of NF1. This study suggests that these changes in growth affect the NF1 patient population as a whole and are not limited to particular subgroups. Our study also confirms the clinical observation that NF1 patients are less likely to be obese than normal populations. The mechanisms by which mutations of the *NF1* gene produce these phenotypic effects are unknown, but understanding how they do so may provide an important clue to the pathogenesis of more serious manifestations of NF1.

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ILLUSTRATIONS

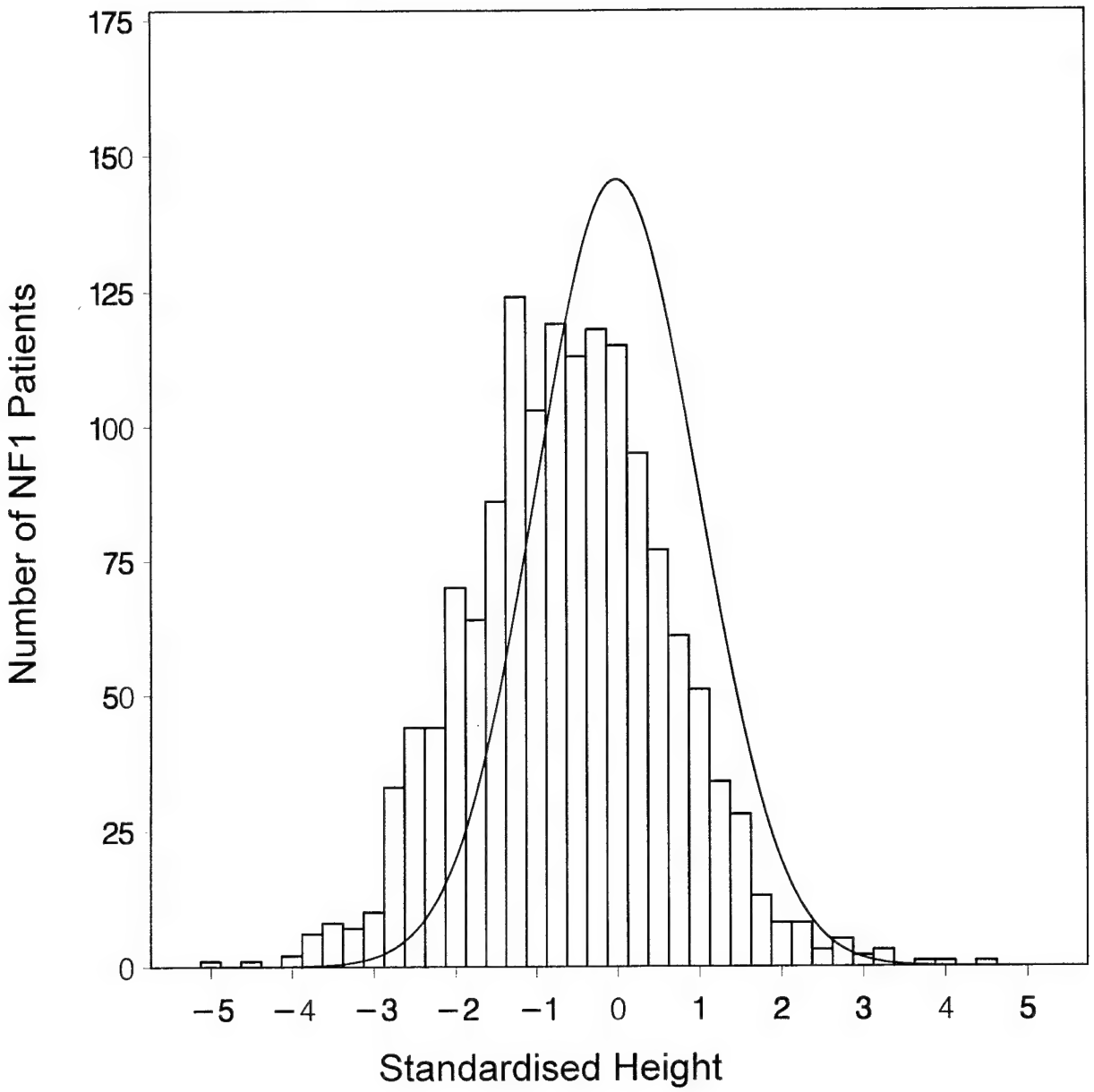
Fig.1: Distribution of sex- and age-standardised height in NF1 patients compared to NCHS norms

Fig.2: Distribution of sex- and age-standardised head circumference in NF1 patients compared to Fels norms

Fig.3: Distribution of sex- and age-standardised head circumference/height in NF1 patients compared to McCammon norms

Fig.4: Body mass index centiles in males 2-40 years compared to NCHS norms

Fig.5: Body mass index centiles in females 2-40 years compared to NCHS norms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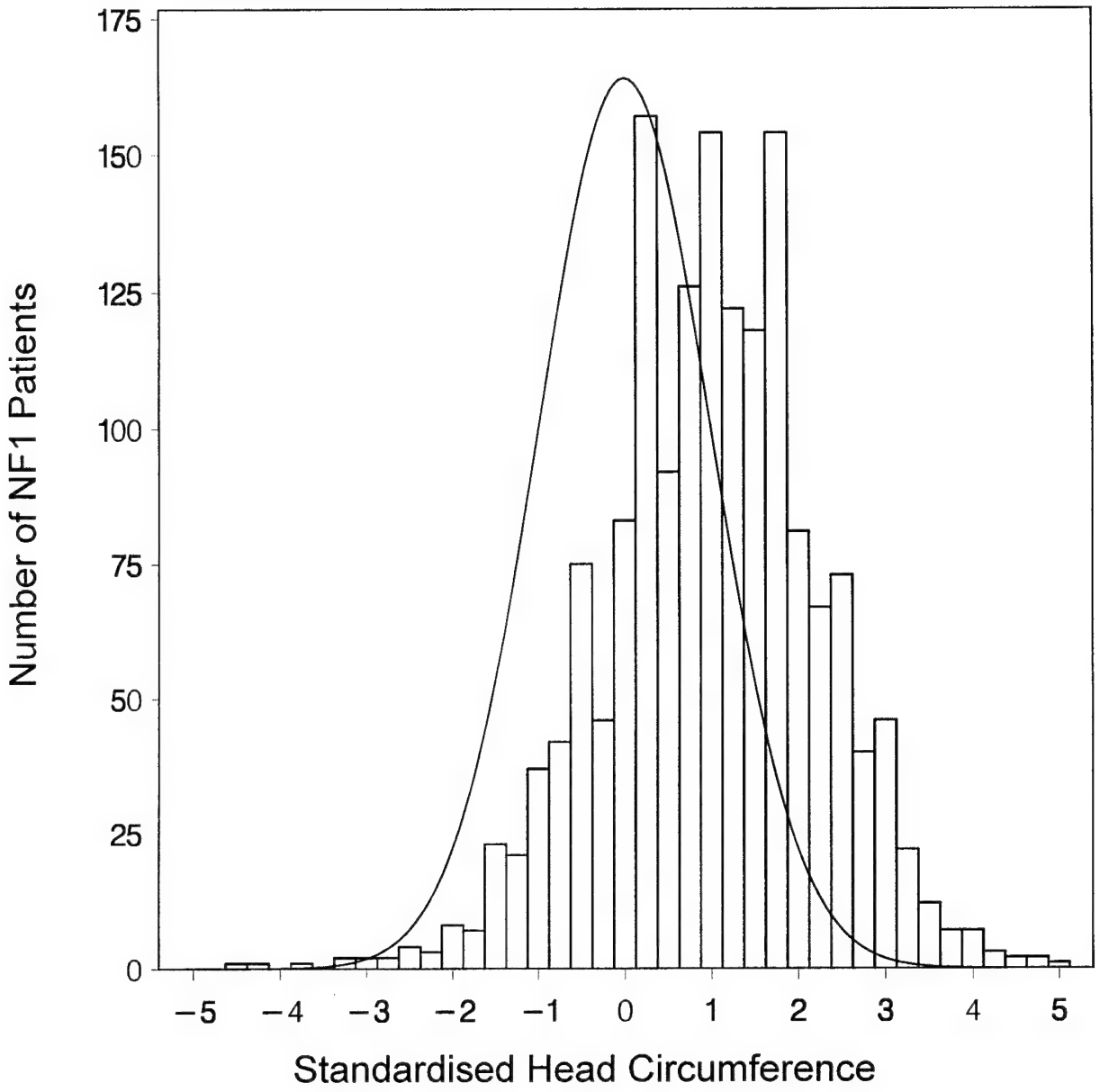
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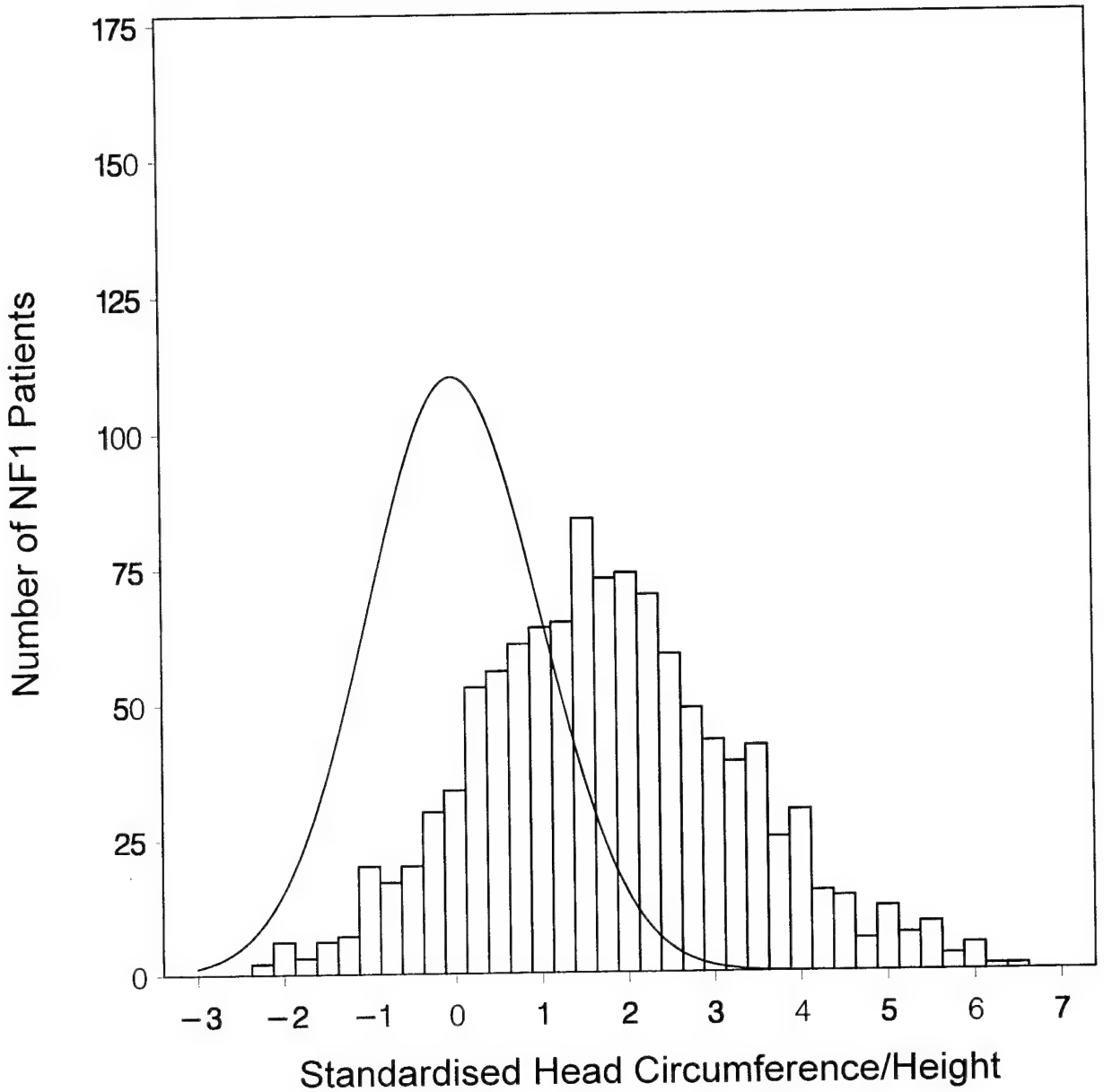
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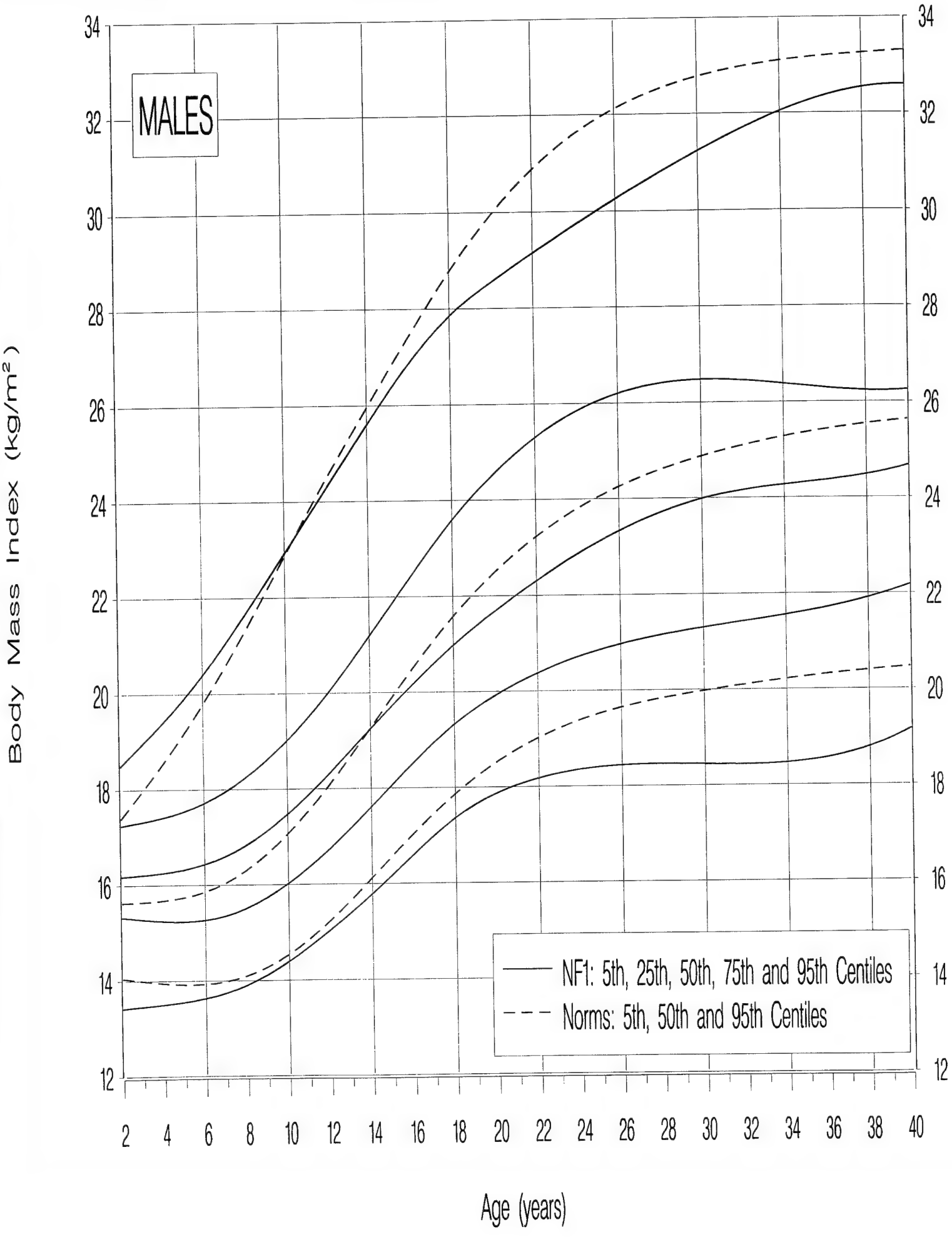
Standardised Head Circumference/Height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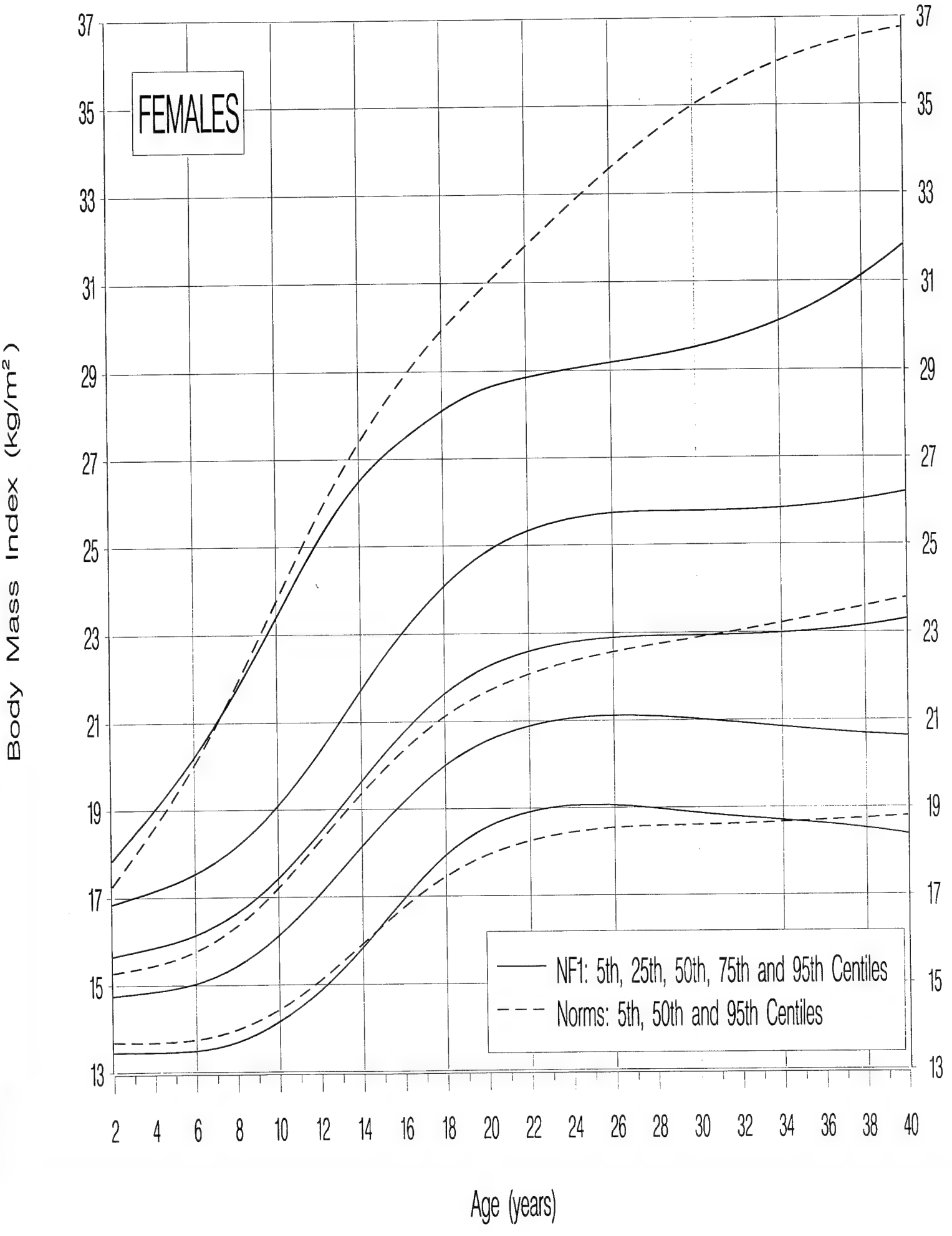
Age (years)
NF1: 5th, 25th, 50th, 75th and 95th Centiles
Norms: 5th, 50th and 95th Centiles


FEMALES
Body Mass Index (kg/m²)
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Age (years)
NF1: 5th, 25th, 50th, 75th and 95th Centiles
Norms: 5th, 50th and 95th Centiles

Growth Curves for Children with Neurofibromatosis 1 (NF1)

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KEY WORDS

head circumference, height, weight, macrocephaly, short stature

ABSTRACT

Objective. Short stature and macrocephaly are more common in children with neurofibromatosis 1 (NF1) than in the general population. The cause of these growth alterations is unknown in most cases, but monitoring growth in affected children assists in detection of complications such as scoliosis, early or delayed puberty or hydrocephalus. We developed standard growth curves for height, weight and head circumference to improve the clinical management of children with NF1.

Methods. Body measurements of children were taken from the National Neurofibromatosis Foundation International Database (NFDB). All children included in this study meet the National Institutes of Health (NIH) Diagnostic Criteria for NF1. Centiles for measurements were plotted alongside those of standard populations.

Results. Children with NF1 are shorter, lighter and have larger heads than unaffected children of similar age. Differences in height and weight, but not head circumference, vary with age between affected and unaffected children.

Conclusions. The growth curves presented here provide a standard for monitoring growth in children with NF1.

KEY WORDS

neurofibromatosis, growth, height, head circumference

INTRODUCTION

NF1 is a progressive autosomal dominant disorder affecting about 1 in 3000 people [1-3]. Its most frequent features are café-au-lait macules, iris Lisch nodules, and discrete and plexiform neurofibromas.

Short stature and macrocephaly are more common in children with NF1 than in the general population, while obesity may be less common among NF1 patients [4]. The cause of the macrocephaly and short stature in most NF1 patients is unknown, but monitoring growth in children with NF1 can help detect complications which affect body measurements, such as scoliosis, early or delayed puberty or hydrocephalus. Both the American Academy of Pediatrics [5] and the National Neurofibromatosis Foundation [6] management guidelines for children with NF1 emphasise the importance of monitoring growth carefully.

We have used data from a large, cross-sectional database to develop standard growth curves for height, weight and head circumference in NF1 patients from 3 months to 18 years of age. These growth charts are useful for the clinical management of children with NF1.

PATIENTS AND METHODS

Definition of Terms

We define "height" as recumbent length in children under 2.25 years of age and as stature in patients 2.25 years of age and over. "Head circumference" is defined as the fronto-occipital circumference.

Patients

All patients included in this study currently meet the NIH Diagnostic Criteria for NF1 [7, 8]. Patient measurements of height, weight and head circumference were obtained from the NFDB [9]. At the time of this analysis, the NFDB included extensive demographic and cross-sectional clinical information on 1959 NF1 patients between 3 months and 18 years of age from 26 participating centres in North America, Europe, Japan and Australia. 92% of the cases are Caucasian, 3% Asian, 2% African-American, and 2% Latin-American. Information was collected and recorded on each patient using a standard procedure.

Only measurements from each patient's first clinical visit were included in the analysis. Patients who were known to have pseudarthrosis, early (often an indicator of optic glioma [10]) or delayed puberty, scoliosis, vertebral dysplasia, or spinal compression were excluded from analyses involving height. Patients with plexiform neurofibroma of the head, early or delayed puberty, or hydrocephalus were excluded from analyses involving head circumference. Patients with either early or delayed puberty were excluded from analyses of weight.

Each participating specialised NF1 centre obtained its measurements by methods that were standard for that centre; the methods and apparatus used may have differed somewhat among centres. We therefore tested the data for each measurement by analysis of variance to determine if significant differences exist among the measurements made by the major contributing centres. Measurements were standardized to allow simultaneous comparison of all age groups. The height and head circumference of each patient were converted into z-scores above or below age- and sex-matched reference population norms. Weight could not be standardised and analysed in this way because it is not normally distributed.

Reference Populations

Standard population norms for height and weight by age were obtained from the National Center for Health Statistics (NCHS) studies [11, 12]. These standards are based on a sample consisting of 15 percent Blacks and 85 percent Whites living in the United States. Standard population norms for head circumference by age were obtained from the Fels Institute study [11]. The Fels Institute data are based on serial measurements of White children.

Growth Curves

NF1 patients were divided into sex and age groups whose medians corresponded to those of the curves used for population norms. A typical series of medians consisted of: 3, 6, and 9 months and 1, 1.5, 2, 2.5, 3…18 years. Age-group limits were determined by splitting the difference between a given median and the next lowest and highest

medians. Patients with ages equidistant from two medians were assigned to the older age group. For example, the 1.5 year-old group included patients from 1.25 to 1.749 years old. The 5th, 25th, 50th, 75th and 95th centiles for height, weight and head circumference were determined directly from the data for each sex in each age group of NF1 patients and plotted on the same graphs as the corresponding centiles for the population standards. The data were plotted and smoothed using SAS [13] by producing a cubic spline that minimises a linear combination of the sum of squares of the residuals of fit and the integral of the square of the second derivative [13, 14]. Smoothed curves were inspected to ensure that the final results reasonably represent the data. Splining was also used by Hamill et al. [11] to generate smoothed standard curves for the NCHS.

RESULTS

Analysis of variance for heterogeneity among major contributing centres of the NFDB revealed no difference ($p=0.21$) between age-standardised heights for 1959 children between 3 months and 18 years of age with NF1. There was significant heterogeneity ($p=0.03$) among major centres with respect to age-standardised head circumference measurements for these children; however, the mean z-score for each centre examined ranged between 0.7 and 0.9.

Height, weight, and head circumference charts for boys and girls with NF1 from 3 to 36 months of age are shown in figures 1 and 2. Figures 3 and 4 provide height, weight, and head circumference charts for boys and girls with NF1 from 2 to 18 years of age. The solid lines in each chart represent the 5th, 25th, 50th, 75th and 95th centiles for children with NF1. The dashed lines represent 5th, 50th and 95th centiles for the reference population.

There are several differences between the growth of children with NF1 and the reference population. The median heights of boys and girls with NF1 3-36 months old are 1cm or less below those for population standards of similar age (figs. 1a and 2a). The median and 5th centile heights of boys and girls with NF1 2-18 years old are also below the reference population (figs. 3a and 4a). This difference appears to increase around the time of puberty.

Most children with NF1 weigh less than their unaffected cohorts from the 3rd to the 18th months of life (figs. 1b and 2b). By the time they reach 18 months of age, however, the weights of affected children are similar to the weights of unaffected

children of the same gender and age. Parity is maintained until around age 9 for girls and age 11 for boys (figs. 3b and 4b). After these ages, the weights of most affected children are lower than those of unaffected cohorts.

The head circumferences of children with NF1 are usually larger than those of unaffected children at 3 months of age and remain relatively large throughout childhood and adolescence (figs. 1c-4c). About 75% of affected children are at or above the standard population median in most age groups.

DISCUSSION

Body measurements may vary between different races and within the same race over different geographic locations [15, 16]. Patient measurements in the NFDB were contributed by centres from around the world. We were concerned that differences between centres would increase the variability of our sample and diminish the usefulness of these growth curves. However, analysis of variance detected no large differences for height and head circumference between the contributing centres.

The contributors to the NFDB are specialised NF1 clinics, and the patients that visit them may be, on average, more severely affected than NF1 patients in general. Height, weight and head circumference are not among the cardinal features by which NF1 is diagnosed [7], and patients with disease features that may influence these measurements have been excluded from our sample. Therefore, it is unlikely that the group of children we studied differs greatly with respect to their height, weight and head circumference from the NF1 population as a whole.

Longitudinal studies of growth are generally preferable to cross-sectional ones. By observing a cohort over several years, longitudinal studies minimise the variation resulting from variables other than age. Cross sectional studies, which observe a different group of people for each age category, are associated with greater variation between age groups. We did not have available sufficient longitudinal data on children with NF1 to develop longitudinal growth standards for this population. We were, however, able to compile cross-sectional data on a large group of children with NF1 at each age. The variation that we observed in growth measurements in this large group of children with

NF1 does not generally appear excessive in comparison to the variability seen in normal populations.

The NCHS and Fels standards were used for comparison to this sample because they cover a wide range of ages. These studies were longitudinal and therefore represent growth more accurately than cross-sectional studies. In addition, they are commonly used in growth charts for routine pediatric care. Although the NCHS and Fels samples do not represent exactly the same racial distributions as the NFDB, race alone is unlikely to account for the differences observed between our curves and the standard curves [4, 11].

Our data were drawn from North America, Europe, Japan and Australia and, therefore, represent a composite of races and regions. One should consider known characteristics of the population from which the patient is drawn when interpreting the plots for an individual child.

The only large study of growth in children with NF1 that has previously been published is that of Riccardi [4]. There are several differences between our study and Riccardi's. Our sample size is four times larger, which makes this the largest study of growth in children with NF1 ever published. Our study provides centile curves calculated directly from the data for height, weight and head circumference in NF1 patients. Riccardi gave means and standard errors for height and head circumference only. Our sample is more homogenous than the Riccardi sample which is 74% White, 15 % Black and 11% Hispanic. All of our patients meet the NIH Diagnostic Criteria for NF1 [7, 8]. Riccardi's study was completed before the NIH Criteria were developed. Although it is likely that almost all of his patients had NF1, some may not have done so.

The results of the two studies are similar in spite of their design differences. Affected children in the Riccardi study are, overall, slightly shorter than those from this study in both sexes and in all age categories, but the standard errors of the means from the Riccardi study overlap the medians from this study. The head circumference means from the Riccardi study do not appear different from the medians in our study. Both are consistently around the 75th centile of the unaffected Fels Institute cohorts in both sexes and most age categories. The same reference populations were used for both studies.

Curves from standard populations provide a useful reference to follow growth in children. However, once the diagnosis of NF1 has been made in a patient, growth curves specific to NF1 are more useful for detection of deviation from the expected growth for that child. Deviation of a child's growth from the NF1 standards may indicate the effect of a specific disease feature such as scoliosis, early or delayed puberty or hydrocephalus. The NF1 curves may also provide reassurance to families and their physicians if an affected child's growth, although "abnormal" for an unaffected child, is actually normal for NF1. The growth curves presented here provide an appropriate standard for monitoring growth in children with NF1.

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ILLUSTRATIONS

Fig. 1(a): Height vs. Age in Males 3 to 36 months

Fig. 1(b): Weight vs. Age in Males 3 to 36 months

Fig. 1(c): Head Circumference vs. Age in Males 3 to 36 months

Fig. 2(a): Height vs. Age in Females 3 to 36 months

Fig. 2(b): Weight vs. Age in Females 3 to 36 months

Fig. 2(c): Head Circumference vs. Age in Females 3 to 36 months

Fig. 3(a): Height vs. Age in Males 2 to 18 years

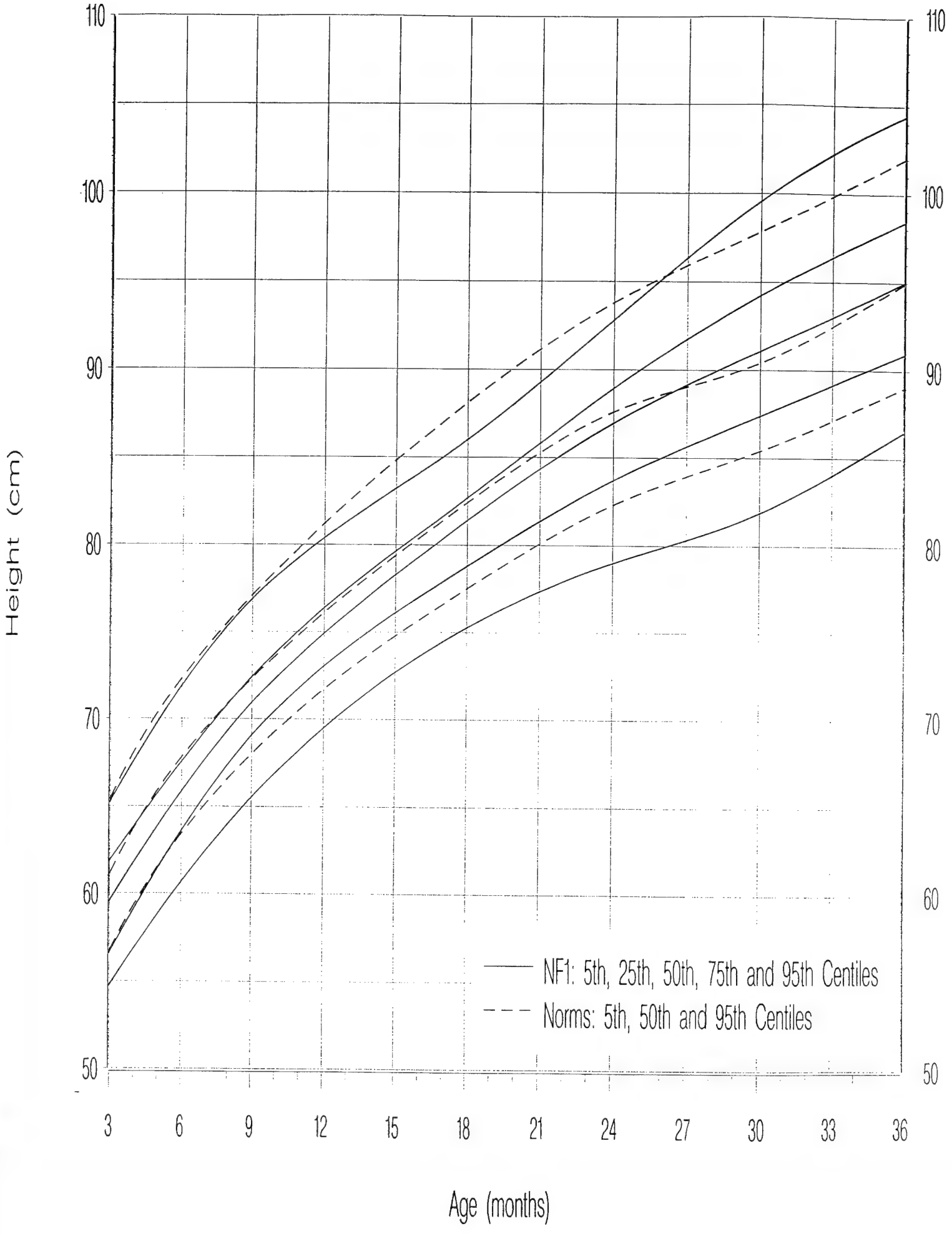
Fig. 3(b): Weight vs. Age in Males 2 to 18 years

Fig. 3(c): Head Circumference vs. Age in Males 2 to 18 years

Fig. 4(a): Height vs. Age in Females 2 to 18 years

Fig. 4(b): Weight vs. Age in Females 2 to 18 years

Fig. 4(c): Head Circumference vs. Age in Females 2 to 18 years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Age (months)
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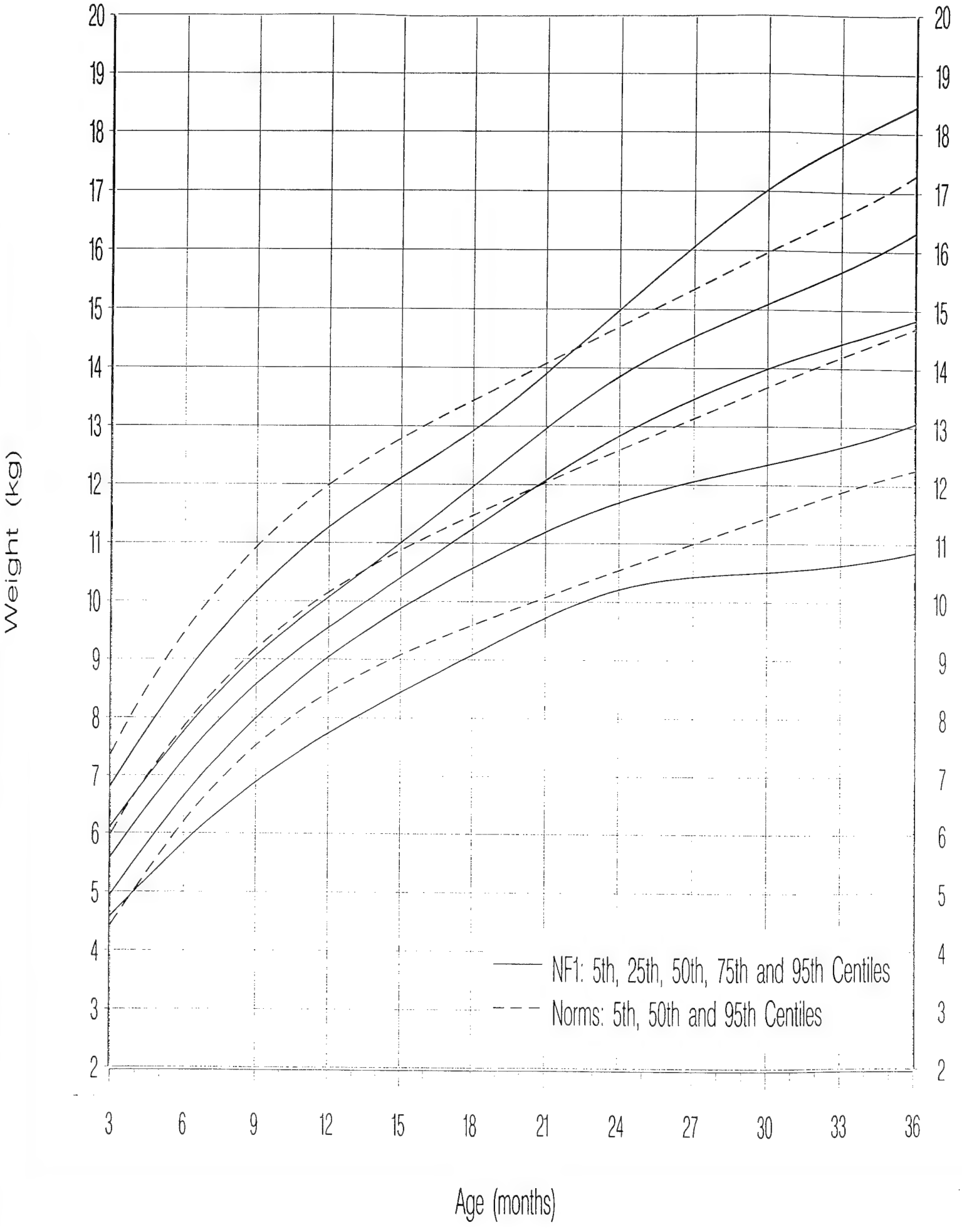
Norms: 5th, 50th and 95th Centiles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Age (months)
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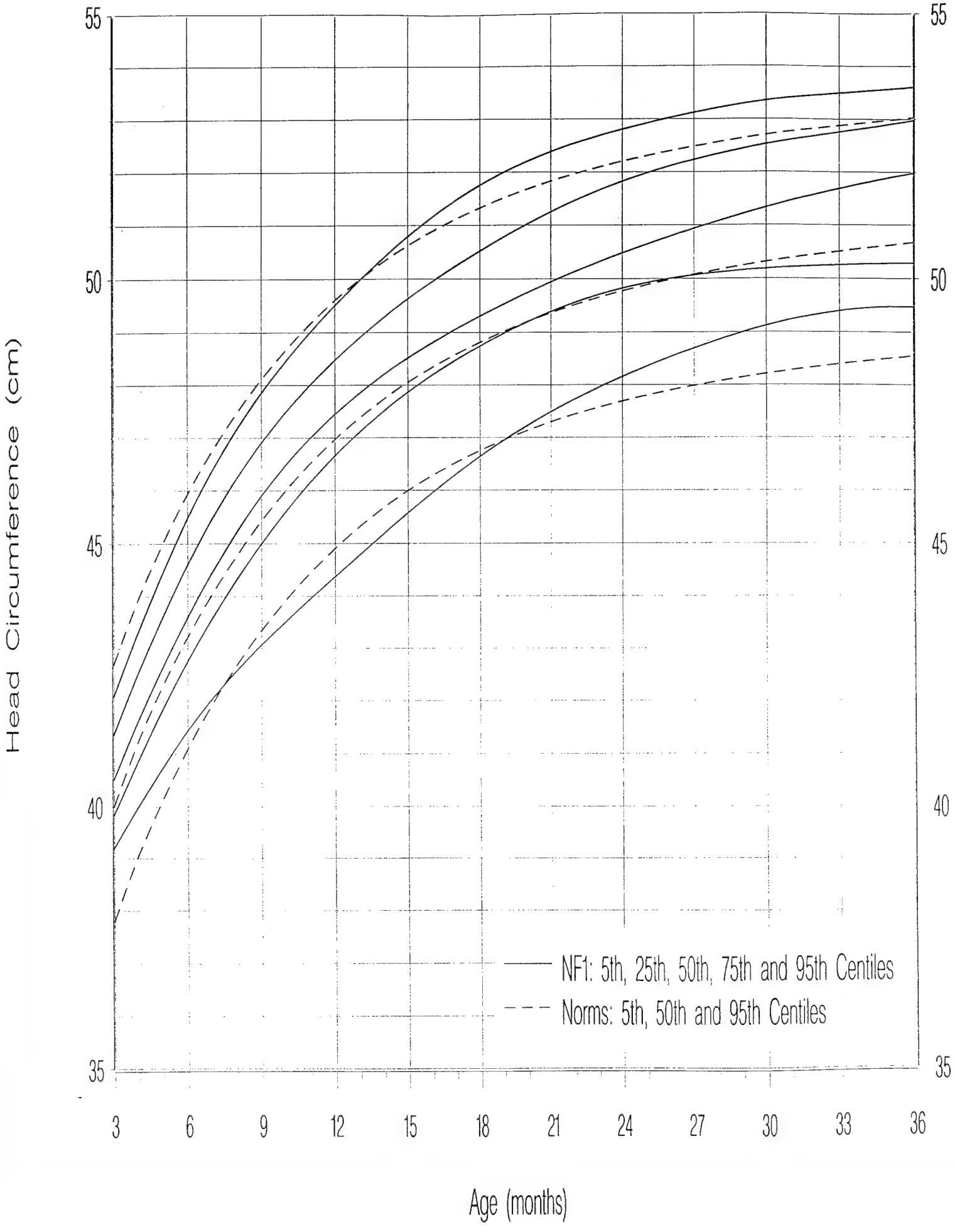
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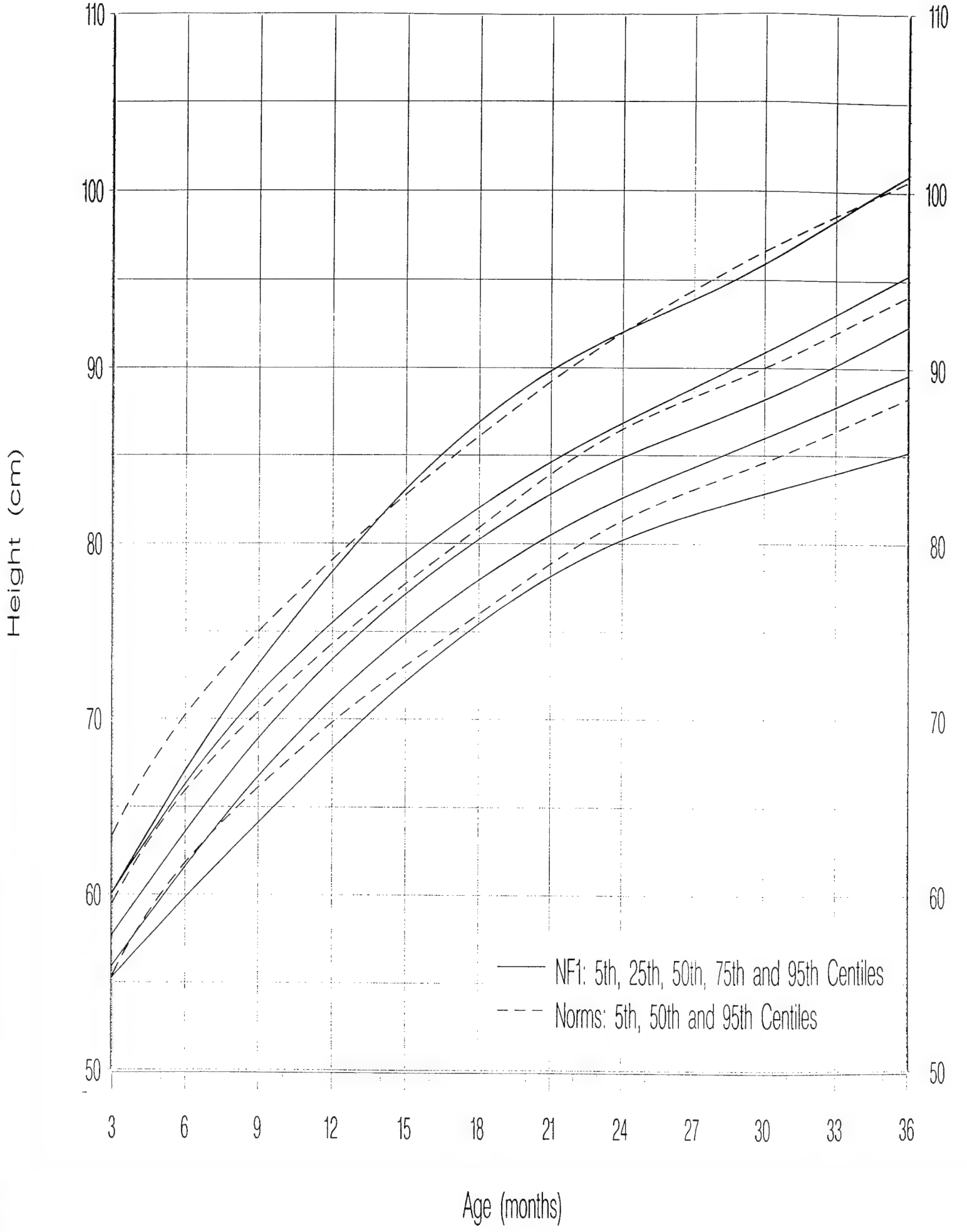
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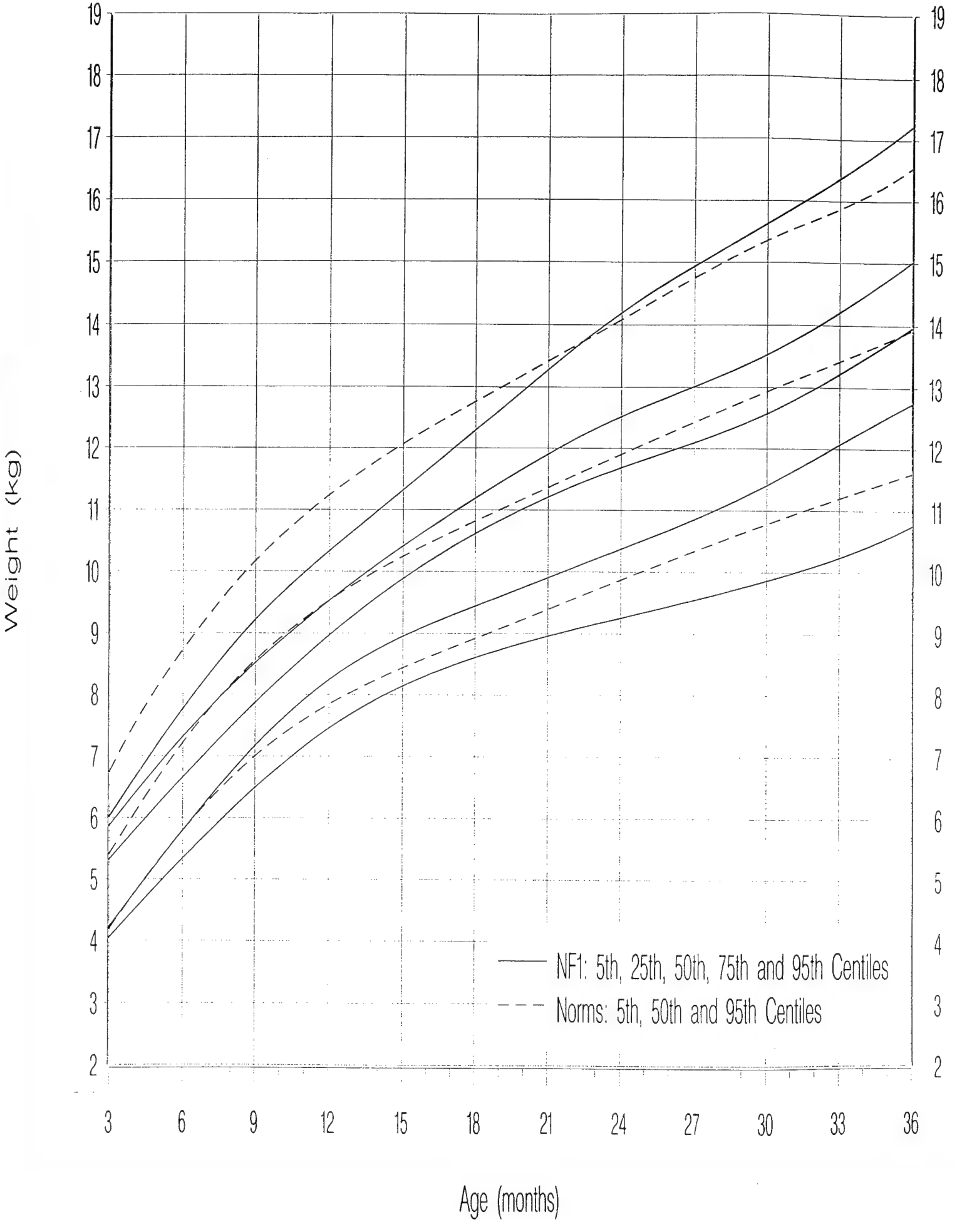
Norms: 5th, 50th and 95th Centiles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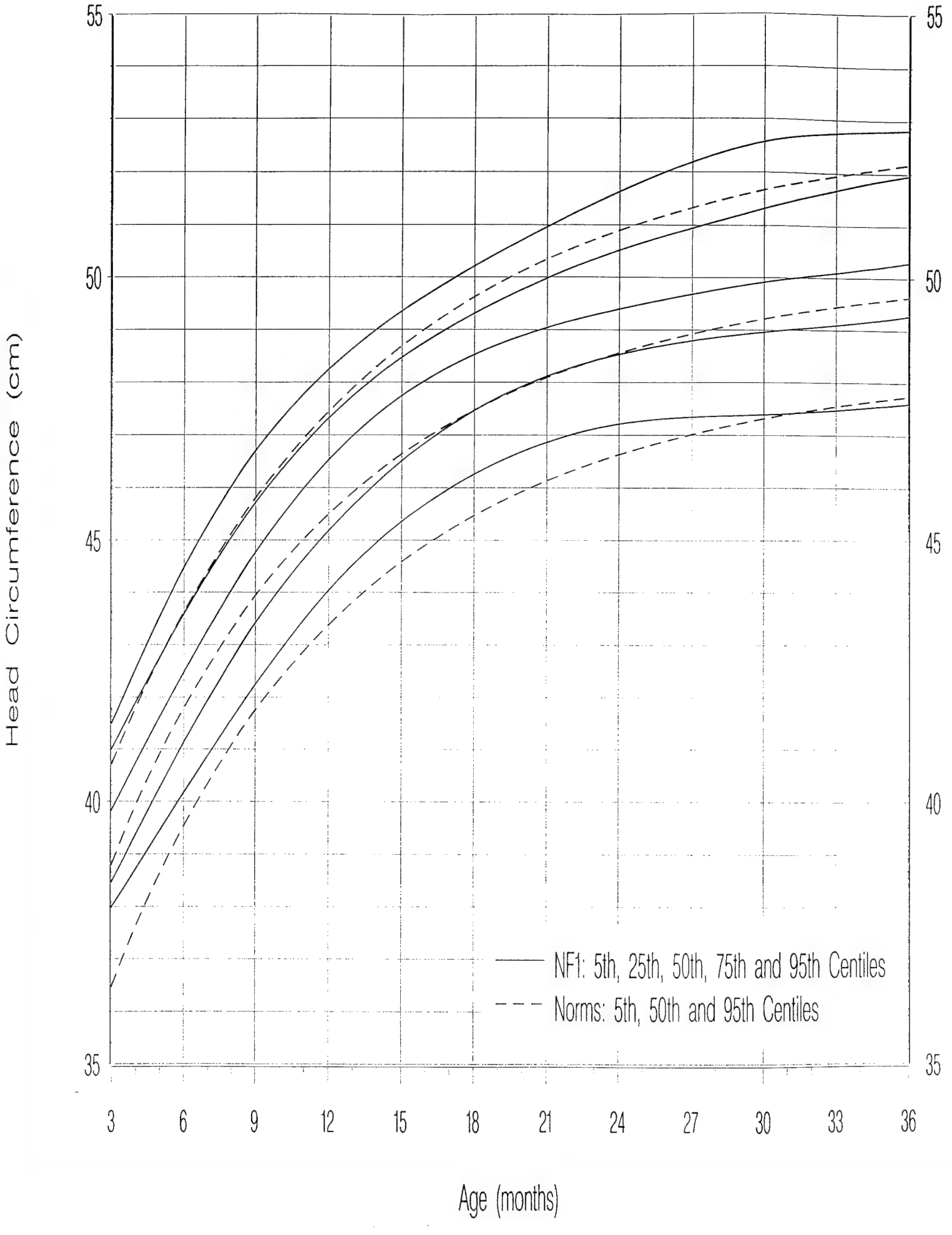
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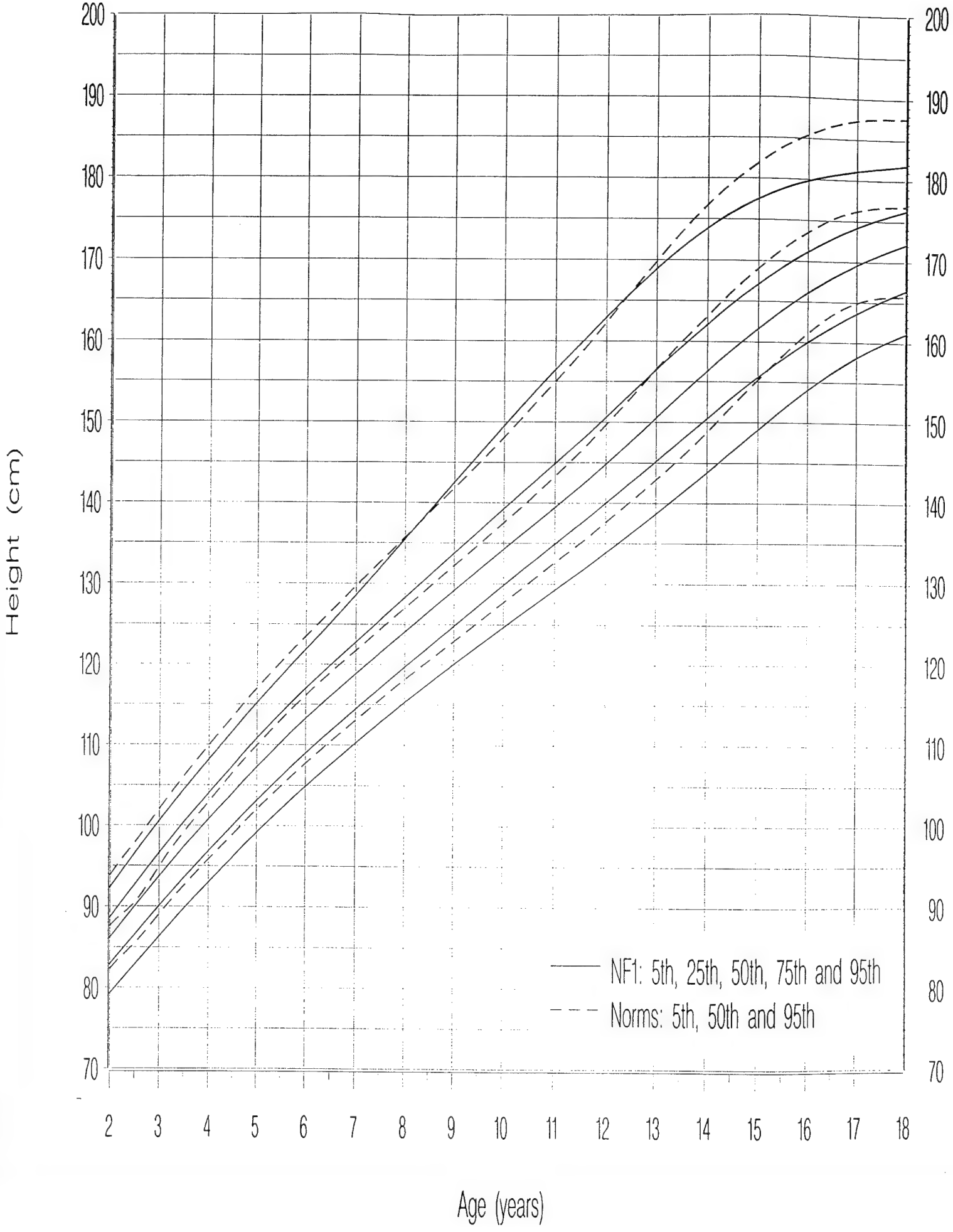
NF1: 5th, 25th, 50th, 75th and 95th Centiles
Norms: 5th, 50th and 95th Centiles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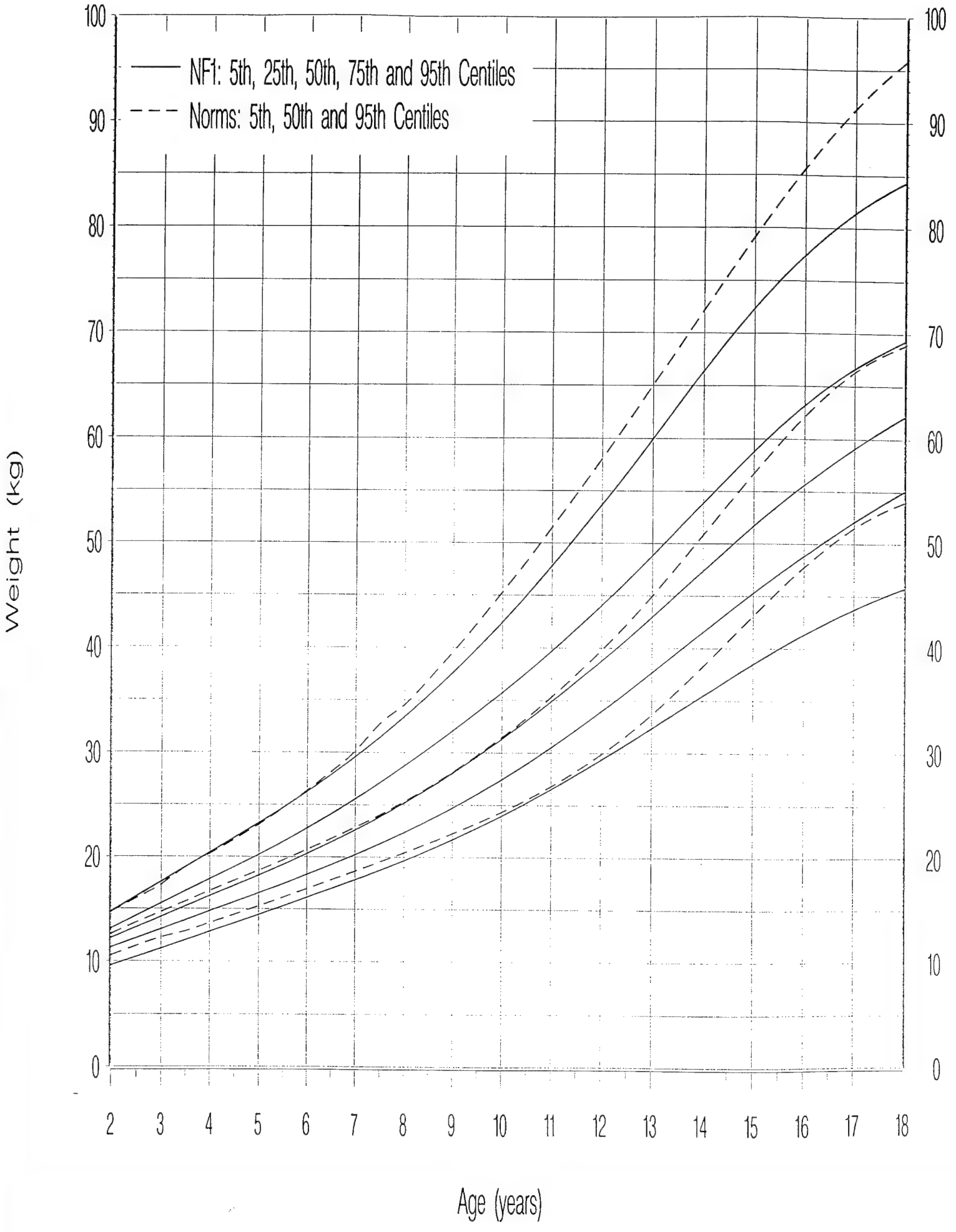
Age (years)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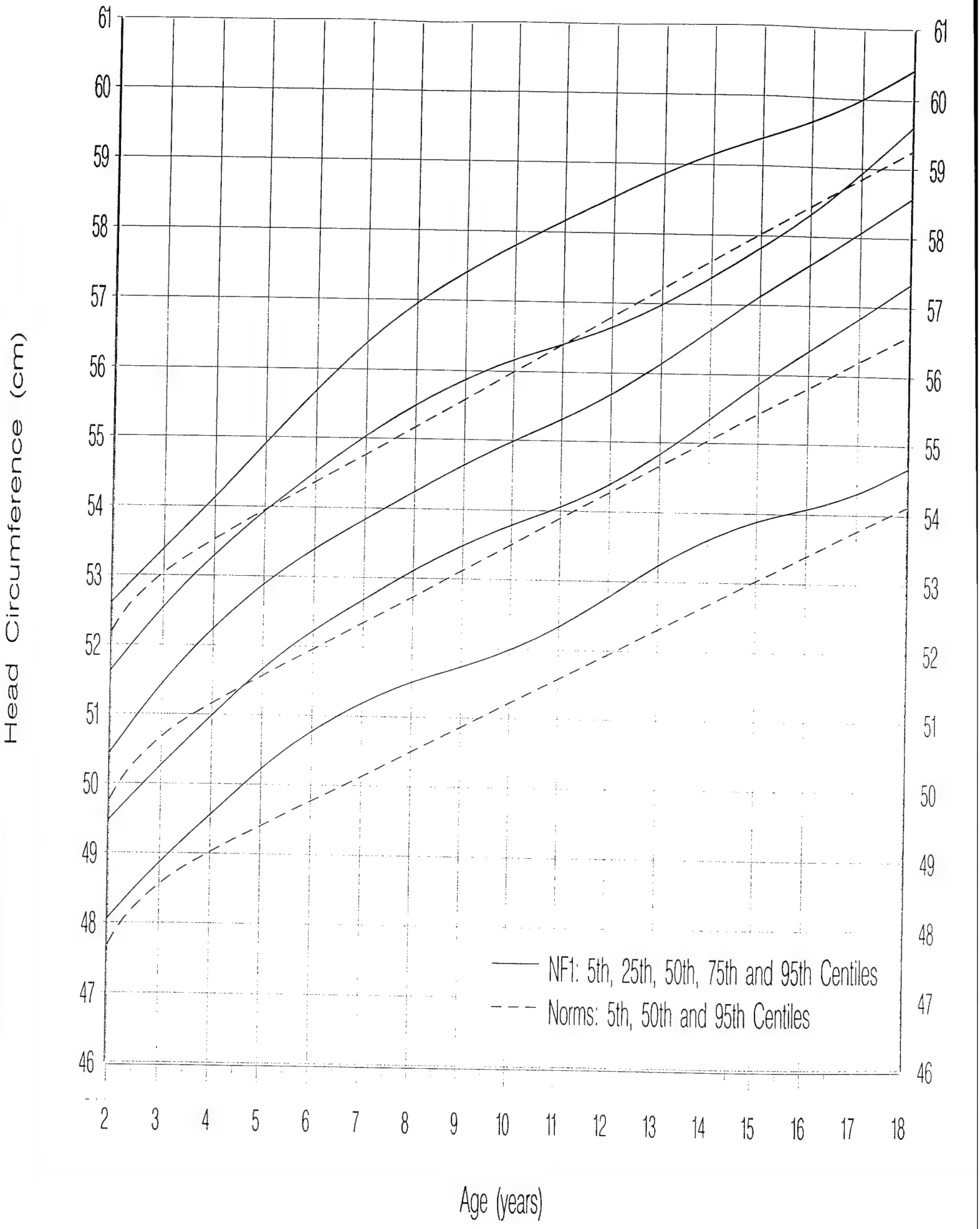
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NF1: 5th, 25th, 50th, 75th and 95th Centiles
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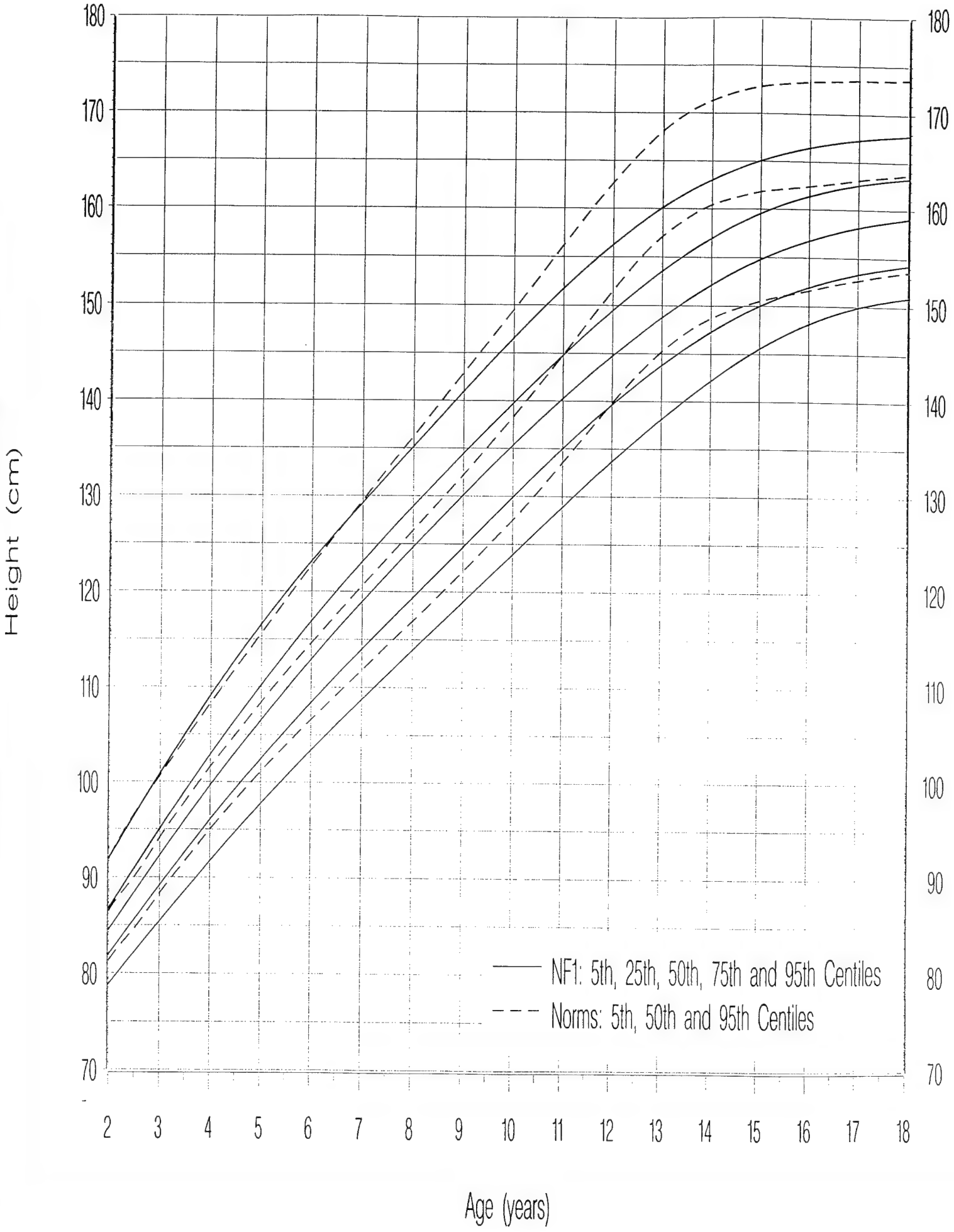
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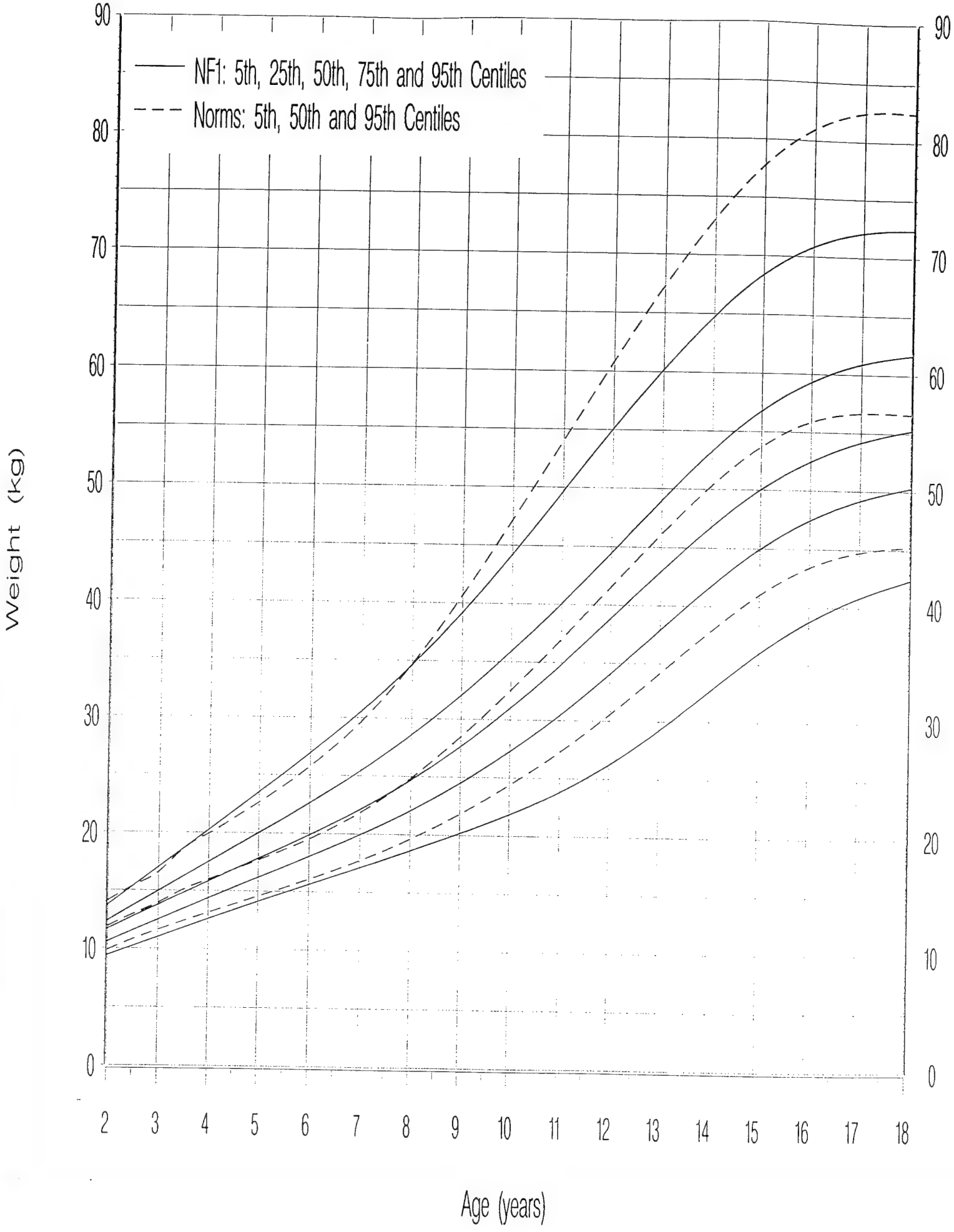
Age (years)
NF1: 5th, 25th, 50th, 75th and 95th Centiles
Norms: 5th, 50th and 95th Centiles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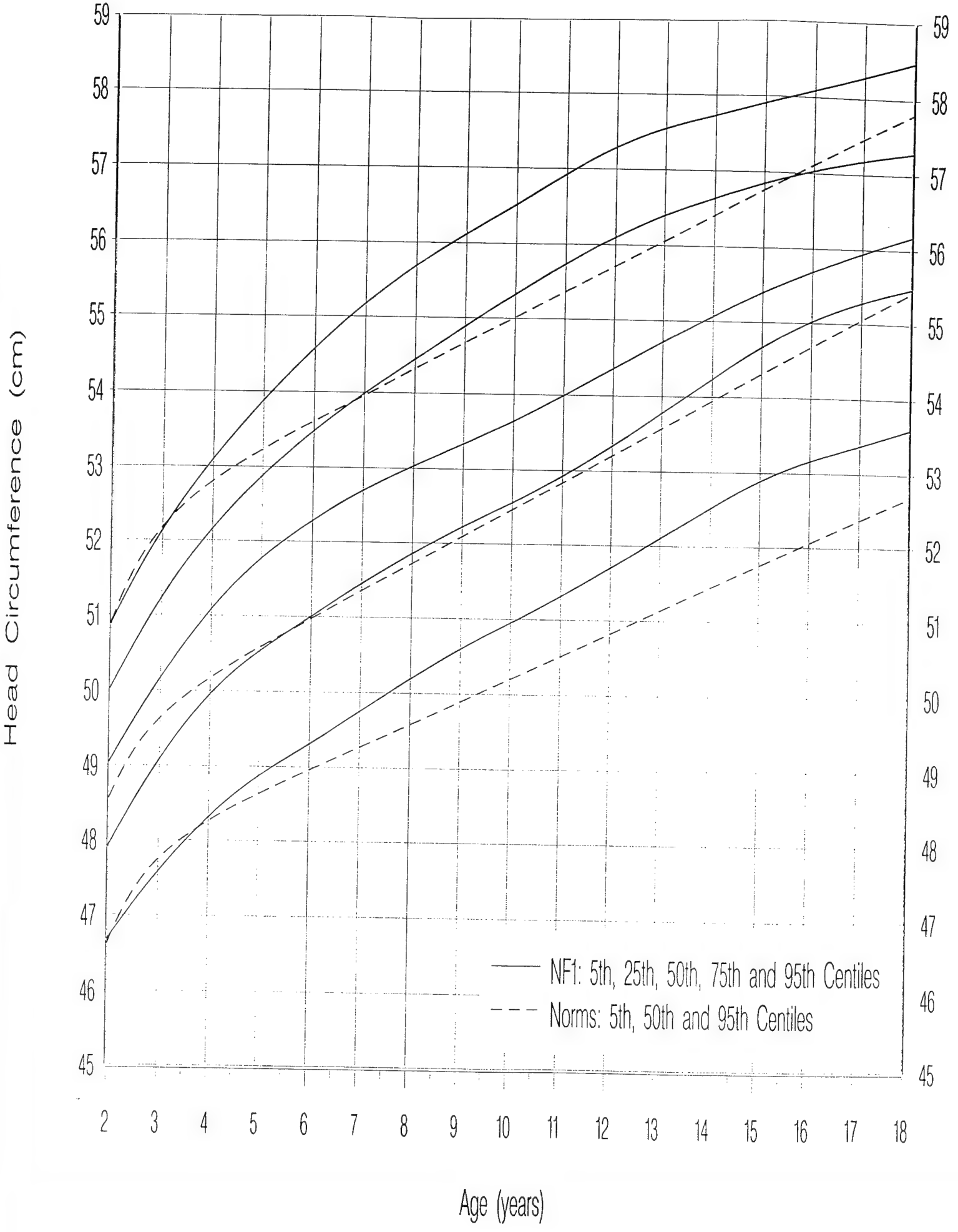
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Age (years)
NF1: 5th, 25th, 50th, 75th and 95th Centiles
Norms: 5th, 50th and 95th Centiles

Associations between Clinical Features of Neurofibromatosis 1 (NF1)

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ABSTRACT

Neurofibromatosis 1 (NF1), an autosomal dominant disease, exhibits extreme clinical variability. This variability greatly increases the burden for affected families and impairs our ability to understand the pathogenesis of NF1. Recognition of clinical heterogeneity within a disease may provide important pathogenic insights, so we tested information from three large sets of NF1 patients for evidence that the clinical features are more likely to occur in some NF1 patients than in others.

Clinical information on 4402 patients with NF1 was obtained from three independent databases. We examined associations between *pairs of clinical features* in individual affected probands. We also examined associations between the occurrence of *individual features* in affected relatives. Associations were summarised as odds ratios with 95% confidence intervals.

We found associations between several *pairs of features* in affected probands: intertriginous freckling and Lisch nodules; discrete neurofibromas and plexiform neurofibromas; discrete neurofibromas and Lisch nodules; plexiform neurofibromas and scoliosis; learning disability or mental retardation and seizures. We also found associations between the occurrence of Lisch nodules, macrocephaly, short stature, and learning disability or mental retardation as *individual features* in affected relatives.

Our observations suggest that, contrary to established belief, some NF1 patients are more likely than others to develop particular manifestations of the disease. Genetic factors appear to determine the development of particular phenotypic features.

KEY WORDS

NF1, database, phenotype, association, familial

INTRODUCTION

Neurofibromatosis 1 (NF1) is a progressive autosomal dominant disorder affecting about 1 in 3000 people (Crowe et al. 1956; Huson et al. 1989; Littler and Morton, 1990). Its most frequent features include café-au-lait macules, Lisch nodules, discrete and plexiform neurofibromas, and learning disabilities. NF1 has been recognized clinically for over 100 years (Von Recklinghausen, 1882), but its natural history is not completely characterized and its pathogenesis is incompletely understood. The disease is fully penetrant, but expressivity is variable (Riccardi, 1992). This variability confounds clinical management and genetic counseling.

The *NF1* gene, identified and sequenced eight years ago, is the second largest known human gene (Cawthon et al. 1990; Viskochil et al. 1990; Wallace et al. 1990). Owing to its size, the lack of clustering of mutation sites, and the fact that recurrent mutations are uncommon, *NF1* gene mutations have been fully characterized in fewer than 300 patients (NNFF, 1999). NF1, therefore, remains a clinical diagnosis that is based on the presence of two or more characteristic physical signs (NIH, 1988; Gutmann et al. 1997).

Manifestations of NF1 vary at different times in an individual's life (Riccardi, 1992; Fitzpatrick et al. 1983; Moritz and Sneider, 1962; Dugoff and Sujanksy, 1996; Knight and Reiner, 1983). Substantial variability also exists among affected members of a single family (Crowe et al. 1956; Zoller et al. 1995). Nevertheless, there is evidence that related individuals with NF1 are more similar to each other than to unrelated affected individuals. Easton et al. (1993) found evidence of intra-familial correlations in the number of café-au-lait macules and neurofibromas and in the presence or absence of

optic gliomas, scoliosis, seizures and referral for remedial education. There are also families in which an unusual phenotype imparted by an *NF1* mutation appears to "breed true". For example, in families with the Watson syndrome variant of NF1, affected relatives all have features of Watson syndrome rather than typical NF1 (Upadhyaya et al. 1990; Allanson et al. 1991). In other families, mutations of the *NF1* locus appear to be expressed consistently as multiple café-au-lait spots without other manifestations of NF1 (Abeliovich et al. 1995) or with spinal neurofibromas in multiple generations (Pulst et al. 1991; Poyhonen et al. 1997; Ars et al. 1998).

Recognition of clinical heterogeneity within a disease may provide important pathogenic insights. For example, understanding that NF1 and NF2 are different diseases (Riccardi, 1982) was a seminal contribution. In order to determine if clinical heterogeneity exists within NF1 itself, we tested three large clinical data sets for associations between the occurrence of different clinical features in individual patients. We also tested for the association of particular features in affected relatives to look for evidence of genetic determinants of clinical variability in NF1. We found consistent associations among the occurrence of different clinical features in individual patients and between the occurrence of the same feature in relatives. Our observations suggest that, contrary to established belief (Riccardi, 1992; Bernhart and Halperin, 1990), some NF1 patients are more likely than others to develop particular manifestations of the disease.

SUBJECTS AND METHODS

Patients and Data Description

All patients included in this analysis were diagnosed with NF1 according to established clinical criteria (NIH, 1988; Gutmann et al. 1997). The study was performed using clinical data from three independent sets of NF1 patients. At the time of this analysis, the National Neurofibromatosis Foundation International Database (NFDB) (Friedman and Birch, 1997) contained descriptions of 2509 NF1 probands, 211 affected parents and 289 of their affected children. The Neurofibromatosis Institute Database (NFID) (Riccardi, 1992) includes standard clinical information on 774 NF1 probands, 132 affected parents and 189 of their affected children. The Manchester NF1 database (MANF1) (McGaughran et al. 1999) includes clinical information on 270 probands, 94 affected parents and 140 of their affected children. There is no overlap among the patients included in these three databases. Specific *NF1* mutations have been identified by molecular analysis in fewer than 1% of these patients.

Statistical Analysis

Twelve of the most common or important clinical features of NF1 were selected for inclusion in this study: intertriginous freckling, discrete cutaneous or subcutaneous neurofibromas (referred to as "discrete neurofibromas"), diffuse or nodular plexiform neurofibromas (referred to as "plexiform neurofibromas"), learning disability or mental retardation, Lisch nodules, scoliosis, tibial or other long bone bowing or pseudarthrosis, optic glioma, macrocephaly, short stature, seizures and neoplasms (other than

neurofibromas or optic glioma). Table 1 summarizes the prevalences of the 12 features in the three databases.

Most of these features were identified by physical examination. Discrete neurofibromas were coded as "present" if the subject had two or more cutaneous or subcutaneous neurofibromas. Short stature was coded as "present" if the subject's height was 2 or more standard deviations below the age- and sex-matched population mean. Subjects with pseudarthrosis, early or delayed puberty, scoliosis, vertebral dysplasia, or spinal compression were excluded from analyses involving height. Macrocephaly was coded as "present" if the subject's head circumference was 2 or more standard deviations above the age- and sex- matched population mean. Subjects with plexiform neurofibroma of the head, early or delayed puberty, or hydrocephalus were excluded from analyses involving head circumference. Lisch nodules were diagnosed or excluded by a slit lamp examination. The presence or absence of optic glioma was determined by cranial MRI or CT examination. Only patients who had definite presence or absence of a feature were considered in comparisons involving that feature.

Pair-wise combinations of the presence or absence of these features in probands were analyzed by 2-by-2 tables using SAS (SAS Institute, 1996). The prevalences of many features of NF1 increase with age (Riccardi, 1992; Cnossen et al. 1998). Two features that both increase with age may show a strong association because older patients are likely to have both features and younger patients are likely to have neither. Therefore, patients from each database were first stratified into 5-year age groups to reduce confounding by age. The method of Mantel and Haenszel (1959) was used to estimate the odds ratio with 95% confidence intervals over the age strata. We performed the analyses

in three independent data sets (NFDB, NFID and MANF1) because we expected to observe many associations that reached nominal statistical significance by chance as a result of multiple comparisons. Odds ratios with 95% confidence intervals that excluded 1.0 in at least two of the three databases were considered unlikely to be due to chance alone. The Breslow-Day method (SAS Institute, 1996) was used to test each triad of odds ratios for homogeneity between the three databases. Triads with p-values greater than 0.05 were considered homogenous. The method of Mantel and Haenszel (1959) was used to estimate the summary odds ratio with 95% confidence intervals for all three databases together.

A second analysis included affected relatives. A feature that increases with age may show a strong intra-familial association because the ages of sibs within a family are usually similar. Therefore, we limited our analysis to parents and children, who usually differ in age by at least 20 years. For each of the 12 features, a 2-by-2 table was used to compare the frequency of a given feature in NF1 children of NF1 parents who had the feature to the frequency in children of parents who lacked the feature. Each individual was counted only once. Odds ratios with 95% confidence intervals were calculated for each of the 12 contingency tables in each database. The Breslow-Day method (SAS Institute, 1996) was used to test for homogeneity and the Mantel-Haenszel method (1959) was used to estimate the summary odds ratio.

RESULTS

Associations of Features in Individual Probands

Pair-wise associations between each pair of the 12 clinical features were tested in 2509 NF1 probands from the NFDB, 774 NF1 probands from the NFID and 270 NF1 probands from the MANF1 database. In the NFDB, an odds ratio of 1.0 was excluded from the 95% confidence limits for 26 of 66 associations tested. There were 23 nominally significant positive associations and 3 nominally significant inverse associations. In the NFID, which contains fewer than 1/3 as many cases as the NFDB, an odds ratio of 1.0 was excluded from the 95% confidence limits for 13 of 66 associations tested. Ten of these nominally significant associations were positive. In the MANF1, which is about 1/9 as large as the NFDB, an odds ratio of 1.0 was excluded from the 95% confidence limits for 5 of 55 associations tested. Odds ratios could not be calculated in the remaining 11 associations due to blank cells in the contingency tables. Overall, 6 of 66 tested associations between pairs of features are statistically significant and in the same direction in at least two of the databases (Table 2). Four of these 6 associations are statistically homogenous between the three databases. One statistically significant inverse association was observed in at least two independent databases. The associations are expressed as odds ratios for each database and as summary odds ratios for all three databases together.

Associations of Features in Relatives

Table 3 summarises the associations for occurrence of the 12 features between: 211 NF1 parents and 289 of their NF1 children from the NFDB; 132 NF1 parents and 189 of their NF1 children from the NFID; and 94 NF1 parents and 140 of their NF1 children pairs from the MANF1. The associations are expressed as odds ratios for each database and as summary odds ratios for all three databases together. Statistically significant positive associations have been shaded. A summary odds ratio of 1 was excluded from the 95% confidence limits 4 of the 12 associations between parents and children. Three of these 4 associations are statistically homogenous between the three databases. No significant negative associations were observed.

DISCUSSION

Associations in Individual NF1 Probands

The large number of cases in these three databases enables us to find significant associations between several common features of NF1 (Table 2). The concordance between the findings in the three independent databases is remarkable. About three (p=.05 multiplied by 66) nominally statistically significant associations were expected by chance in each database, and one would expect chance associations to differ in the NFDB, NFID and MANF1. The reproducibility of our results suggests that these associations are probably not due to chance alone.

The positive associations observed may reflect shared pathogenic mechanisms underlying the associated features. For example, NF1 probands with seizures may be more likely also to have learning disabilities or mental retardation than patients without seizures (Table 2) because the effect of the *NF1* mutation on brain development is greater in patients who have seizures.

The association observed between the occurrence of plexiform and discrete neurofibromas (Table 2) is consistent with the histopathological similarity between these lesions (Harkin and Reed, 1969; Burger and Scheithauer, 1994) and the fact that both are associated with acquired loss or mutation of the normal *NF1* allele in at least some cases (Serra et al. 1997; Sawada et al. 1996). NF1 patients who develop plexiform neurofibromas usually do so during childhood (Riccardi, 1992). In contrast, discrete neurofibromas are uncommon in young children but are almost universally present among adults with NF1. The association we observed much stronger in younger than in

older NF1 patients. The odds ratio was 6.9 among patients under 5 years old, 3.1 among those 5-9, but only 1.3 among those over 40. This raises the interesting possibility that NF1 patients with plexiform neurofibromas develop discrete neurofibromas earlier than patients without plexiform lesions.

The association between Lisch nodules and discrete neurofibromas and intertriginous freckling (Table 2) has been previously reported (Pietruschka, 1961; Zehavi et al. 1986), but the responsible mechanism is unknown. Lisch nodules (Perry and Font, 1982) and freckles (Fitzpatrick, 1981) are derived from cells of melanocytic origin, and all three lesions involve cells derived from the embryonic neural crest (Weston et al. 1981). This is consistent with the suggestion that NF1 is a neurocristopathy (Huson and Hughes, 1994) but does not explain why other neural crest-derived tissues, such as the sympathetic ganglia, thyroid C-cells, and parathyroids, are rarely involved in NF1. Moreover, many of features of NF1, such as learning disabilities, dysplastic scoliosis, and tibial pseudarthrosis, do not appear to be abnormalities of neural-crest derived tissues.

Although plexiform neurofibromas growing near the spine can cause abnormal curvature and result in scoliosis, the association we observed between plexiform neurofibromas and scoliosis does not lose significance when patients with plexiform neurofibromas of the trunk are excluded. Furthermore, two different forms of scoliosis may occur in NF1 patients – a dystrophic form that occurs within the first decade of life and is often severe and rapidly progressive, and a milder form that occurs later and resembles common adolescent scoliosis (Riccardi, 1992). The association we observed involves only early onset scoliosis. The pathogenic basis for this association and for the

association we observed between the absence of pseudarthrosis or bowing and discrete neurofibromas is obscure.

Most of the associations observed among probands in this study are moderate in strength – positive associations generally have odds ratios in the range of 2.0-3.0 and negative associations have odds ratios in the range of 0.3-0.5 (Tables 1 and 2). Such pair-wise associations are not strong enough to be useful clinically for predictive classification of patients. Nevertheless, our observation of similar associations in three independent databases strongly suggests that common disease features do not occur entirely at random in NF1 and that some patients are more likely than others to develop particular features. This interpretation contrasts with the generally held view that any NF1 patient may develop any manifestation of the disease (Riccardi, 1992; Bernhart and Halperin, 1990). Most of the associations we observed have never been noted before. Their identification is an important step towards understanding the pathogenesis of NF1.

Associations between Clinical Features in Parents and Children

Our observations in probands suggest that shared pathogenic mechanisms underlie several common features of NF1. If genetic factors influence these pathogenic mechanisms, one would expect familial aggregation of such features to occur. We, therefore, tested for associations between the occurrence of the 12 features among affected relatives.

The ages of sibs within a family are usually similar, and an associations may be noted because older sib pairs are more likely both to have a feature and younger sib pairs both to lack a feature that increases in prevalence with age. Parents and children usually

differ in age by at least 20 years, so significant associations between the occurrence of a feature in a parent and child are unlikely to be inflated by confounding by age. Due to this age difference, we expect the odds-ratios from parent-child comparisons to yield conservative estimates of intra-familial associations. Consequently, we limited our analysis to affected parents and children.

Several strong associations were found by comparing the presence or absence of the 12 features between affected parents and children (Table 3). Lisch nodules, optic glioma, macrocephaly and short stature were significantly associated and homogenous between the three databases. The association for learning disability or mental retardation was statistically significant but was not homogenous between the three databases. This may be due to regional differences in how the feature is diagnosed. These associations are probably not due to ascertainment bias. The subjects were assessed in specialized NF clinics and parents and children were excluded unless the presence or absence of the feature in question had been determined in both members.

No negative associations were found between affected parents and children. This is consistent with our hypothesis that affected relatives have a more similar NF1 phenotype than unrelated people. The absence of negative associations also supports the statistical validity of our observations. One would expect to observe negative, as well as positive, associations by chance alone due to multiple comparisons.

We observed familial associations for the occurrence of Lisch nodules, macrocephaly, short stature, and learning disability or mental retardation. In a previous study, Easton et al. (1993) examined 175 individuals with NF1 and found evidence of intra-familial correlations in the number of café-au-lait macules and neurofibromas and in

the presence or absence of optic gliomas, scoliosis, seizures and referral for remedial education. Easton observed no correlations for head circumference or plexiform neurofibromas. Furthermore, phenotypic similarity of these NF1 features was found to decrease with decreasing genetic similarity – a trend not examined here. Unlike our study, the results of Easton et al. rely heavily on data from six pairs of monozygotic twins. Nevertheless, the studies are both consistent with genetic factors influencing the phenotypic expression of *NF1* mutations in patients with NF1.

The strong phenotypic similarity among relatives may be evidence of an *NF1* allele-phenotype correlation. Although generally not striking in NF1, phenotypic modification by the nature of the mutant allele has been demonstrated in large deletions of the *NF1* gene, which tend to result in a severe phenotype (Tonsgard et al. 1997). Other genetic factors that might influence the phenotype in NF1 patients include variants of the normal *NF1* allele and "modifying genes" at other loci.

First-degree relatives share half of their DNA sequences at other loci. Similarities at these other loci may contribute to the phenotypic similarities observed in families with NF1. Our findings complement those of Easton et al. (1993) and support their hypothesis that modifying genes influence the NF1 phenotype. The NF1 protein, neurofibromin, is known to interact with many other proteins, including tubulin (Bollag et al. 1993), kinases (Marchuk et al. 1991) and Ras (Xu et al. 1990; Buchberg et al. 1990). Functional variants of these proteins might influence the NF1 phenotype.

Our studies demonstrate that, although the NF1 phenotype is highly variable, some patients are more likely than others to develop certain common disease features. In addition, we show that genetic factors may determine the particular phenotypic features

that develop in many cases. Further clinical, epidemiological, and molecular studies are necessary to elucidate the pathogenesis of this complex disease fully, but our investigations provide hope that some serious complications of NF1 can be predicted or prevented.

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ELECTRONIC DATABASE INFORMATION

NNFF International NF1 Genetic Mutation Analysis Consortium (1999) Available from:

URL:http://www.nf.org/nf1gene/nf1gene.mutdata.summary.html

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Table 1: Prevalence of Clinical Features of NF1 in Probands and Affected Relatives

	NFDB				NFID				MANF1			
	Probands		Affected Relatives		Probands		Affected Relatives		Probands		Affected Relatives	
Clinical Feature	*%*	*(n)*	*%*	*(n)*	*%*	*(n)*	*%*	*(n)*	*%*	*(n)*	*%*	*(n)*
Freckling	82.9	(2420)	77.2	(452)	75.8	(662)	75.0	(148)	90.8	(228)	81.8	(132)
Discrete NFs	52.5	(2499)	51.0	(467)	51.8	(713)	45.8	(168)	76.0	(242)	54.9	(122)
Plexiform NFs	25.8	(2490)	15.2	(467)	41.5	(743)	25.3	(178)	19.3	(270)	15.2	(171)
Lisch nodules	55.9	(1837)	63.4	(339)	83.0	(395)	89.1	(101)	70.1	(174)	62.1	(66)
Optic glioma	25.0	(1000)	16.8	(125)	21.3	(400)	11.7	(77)	9.5	(190)	12.9	(70)
Seizures	6.8	(2509)	4.3	(470)	5.9	(732)	3.8	(185)	3.0	(237)	9.4	(149)
LD/MR	47.2	(1899)	52.1	(355)	51.1	(587)	48.6	(142)	24.1	(187)	30.0	(100)
Pseudarthrosis	5.2	(2497)	3.9	(462)	4.0	(756)	2.1	(189)	2.1	(243)	2.7	(149)
Scoliosis	25.6	(2498)	14.0	(463)	25.0	(645)	23.1	(156)	14.6	(246)	14.7	(150)
Macrocephaly	20.1	(1553)	17.6	(301)	30.8	(598)	25.5	(137)	24.2	(186)	19.1	(115)
Short stature	12.6	(1903)	20.1	(353)	7.3	(605)	4.0	(124)	34.3	(134)	56.3	(80)
Neoplasms	6.6	(2509)	3.8	(470)	10.7	(774)	9.1	(208)	6.5	(230)	5.8	(137)

NFs: neurofibromas
LD/MR: learning disability or mental retardation

Table 2: Odds-ratios with 95% confidence limits for associations of features among age-stratified probands with NF1

Associated Features		NFID	NFID	MANF1	Homogeneity (p)	*Summary Odds-Ratio*
Freckling	Lisch Nodules	1.8 (1.3-2.4)	1.2 (0.6-2.4)	3.2 (1.2-8.6)	0.85	*1.7 (1.3-2.3)*
Discrete NFs	Plexiform NFs	2.8 (2.3-3.6)	1.9 (1.2-2.9)	2.1 (0.7-6.3)	0.11	*2.6 (2.1-3.1)*
Discrete NFs	Lisch Nodules	1.6 (1.3-2.0)	2.6 (1.3-5.5)	1.9 (0.9-4.2)	0.04	*1.7 (1.3-2.1)*
Discrete NFs	Pseudarthrosis	0.5 (0.3-0.9)	0.3 (0.1-0.8)		0.04	*0.6 (0.4-0.8)*
Plexiform NFs	Scoliosis	1.5 (1.2-1.8)	1.8 (1.2-2.6)	1.8 (0.8-4.1)	0.72	*1.6 (1.3-1.9)*
Seizures	LD/MR	1.7 (1.2-2.3)	3.3 (1.5-7.1)	6.5 (1.7-24.6)	0.18	*2.5 (1.8-3.4)*

Odds-ratios with 95% confidence limits could not be determined for comparisons in which contingency tables contained empty cells. The corresponding cells in table 2 are blank.
NFs: neurofibromas
LD/MR: learning disability or mental retardation

Table 3: Odds-ratios with 95% confidence limits for associations of features between parents and children with NF1

	NFDB	NFID	MANF1	Homogeneity (p)	*Summary Odds Ratio*
Freckling	1.9 (0.8-4.7)	0.8 (0.2-3)	1.7 (0.4-7.4)	0.52	*1.5 (.8-2.8)*
Discrete NFs	1.8 (0.9-3.9)	3.4 (0.7-16.1)	0.5 (0.1-2)	0.13	*1.6 (.9-2.9)*
Plexiform NFs	0.8 (0.4-1.9)	0.6 (0.3-1.4)	1.7 (0.6-5.3)	0.35	*0.9 (.5-1.4)*
Lisch Nodules	**8.5 (3.3-22.3)**	5.8 (0.3-100)	**10.5 (1.8-61.2)**	0.94	*8.7 (4.2-18.1)*
Optic Glioma		2.0 (0.3-12.7)	**27.3 (1.9-395)**	0.17	*3.6 (1.0-12.3)*
Seizures	1.2 (0.1-9.3)	7.1 (0.6-84.6)		0.38	*1.7 (.4-7.5)*
LD/MR	1.3 (0.8-2.2)	**5.8 (2-16.6)**	**16.8 (1.8-160)**	0.004	*2.0 (1.3-3.1)*
Pseudarthrosis	5.1 (0.5-49.3)				*1.8 (.2-14.3)*
Scoliosis	1.7 (0.7-3.8)	1.6 (0.6-4.1)	1.3 (0.3-6.6)	0.96	*1.6 (.9-2.8)*
Macrocephaly	**8.2 (2.9-22.7)**	2.0 (0.8-5.2)	2.6 (0.5-12.4)	0.12	*3.5 (1.9-6.2)*
Short Stature	**5.9 (2.3-14.8)**		2.3 (0.6-9.2)	0.47	*4.2 (2.0-8.6)*
Neoplasms	5.0 (1-26.4)		**25.5 (1.3-485)**	0.001	*1.3 (.4-3.9)*

Odds-ratios with 95% confidence limits could not be determined for comparisons in which contingency tables contained empty cells. The corresponding cells in table 3 are blank.

NFs: neurofibromas

LD/MR: learning disability or mental retardation

Cardiovascular Malformations and other Cardiac Abnormalities in Neurofibromatosis 1 (NF1)

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Headers: Lin et al., NF1 and cardiac abnormalities

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Although it is well-recognized that a peripheral vasculopathy exists in neurofibromatosis 1 (NF1), it is unclear whether intracardiac abnormalities are more common. We reviewed the frequency of cardiac abnormalities, in particular, cardiovascular malformations (CVMs), among 2550 patients with NF1 in the National Neurofibromatosis Foundation International Database from 1991-1998. Ninety-seven patients who fulfilled the NIH diagnostic criteria and were reported as having a cardiac abnormality comprised the main study group. We excluded 15 patients (11 were less than 10 years old) with provisional NF1 in whom the NIH criteria were incompletely fulfilled.

Of the 97 verified patients, CVMs were reported in 50/2,550 (2.0%). This frequency would be reduced somewhat by excluding 8 patients in whom the CVM diagnosis was called "possible" or inadequately specified (42/2,550, 1.6%). In 15 (36%) of these 42 patients with a CVM, the diagnosis was confirmed by echocardiography, catheterization or surgery. A mechanistic classification was used to classify the CVMs [Clark, 1995]. There was a predominance of Class II "flow" defects (40/50, 80%), typically pulmonic stenosis (22/40, 54%). Of interest was the paucity of Class I conotruncal defects (1/50, 4%), and the absence of Class IV (atrioventricular canal), complex single ventricle and laterality defects.

In addition to the 50 patients with a CVM, 5 patients were reported as having the Watson or NF1-Noonan syndrome. Not surprisingly, three had valvar pulmonic stenosis; one each had mitral valve prolapse and a heart murmur. Besides the 50 patients with CVMs, there were 26 patients with other cardiac abnormalities (15 with unspecified murmur, 5 with mitral valve prolapse, 1 with intracardiac tumor, 5 with electrocardiogram abnormalities). No patient in this study had hypertrophic cardiomyopathy (HCM). There

were 16 patients with peripheral vascular abnormalities (and an additional 4 patients among those who also had a CVM discussed above).

In NF1 patients without the Watson or NF1-Noonan syndrome, there appears to be a greater than expected frequency of CVMs. Although the overall frequency is not striking, the predominance of pulmonic stenosis, usually valvar, and the relative absence of more complex CVMs including conotruncal CVMs, suggests mechanisms besides neural crest maldevelopment. This study was not able to answer the question of whether HCM in NF1 is a real or coincidental association. The familiar association of peripheral vascular abnormalities, especially renal artery stenosis, was observed.

KEY WORDS: cardiovascular malformation; congenital heart defect; database; hypertrophic cardiomyopathy; neurofibromatosis type 1; NF1; NF1-Noonan syndrome; peripheral arterial stenosis; pulmonic stenosis; Watson syndrome

INTRODUCTION

Neurofibromatosis 1 (NF1) is a distinctive autosomal dominant disorder (MIM #162200) with protean manifestations [Rubenstein and Korf, 1990; Riccardi, 1992; Huson and Hughes, 1994]. The type and frequency of cardiovascular abnormalities is unclear, although it is well-accepted that NF1 includes a vasculopathy consisting of peripheral arterial stenoses and ectasias [Friedman, 1999].

The type and incidence of cardiovascular malformations (CVMs) has not been well-defined [Lin and Garver, 1988; Friedman and Birch, 1997; Friedman, 1999]. The frequency of CVMs in 8 large series of NF1 patients ranged from 0.4 - 6.4% (Table 1). Often, it was unclear whether the reported patients had verifiable NF1, a bona fide CVM, or a dysmorphic syndrome (Watson, NF1-Noonan). Despite the vagaries of reporting, there was a predominance of pulmonic stenosis, often valvar. Likewise, case reports and small series also include pulmonic stenosis. The single "NF" patient with a complex CVM [Stoll et al., 1995] did not have NF1 substantiated and may have had a multiple congenital anomaly syndrome related to parental consanguinity.

Less frequent than CVMs are reports of hypertrophic cardiomyopathy (HCM) in NF1 patients (Table II). In 2 cases, it is likely that HCM and NF1 were segregating as independent autosomal dominant traits [Wille et al, 1980]. Other issues clouding the interpretation of these reports include likely extrinsic neurofibroma compression causing biventricular hypertrophy [Pung and Hirsch, 1995], and possible NF1-Noonan syndrome [Gerbaux et al., 1974]. The hypertrophy was usually described as left ventricular and often, subaortic ("idiopathic hypertrophic subaortic stenosis"). Two patients had either post-mortem examination [Sachs et al., 1984] or biopsy [Schrader et al., 1986] with myocardial examination showing non-specific

changes and fiber disproportion with fibrosis, respectively. It remains unclear whether CVM or HCM represent an occasional manifestation of the NF1 mutation, or abnormalities which occur together as a matter of chance. There is no debate that rare reports of intra- or extracardiac neurofibromas are part of NF1 itself, although they can mimic a CVM or HCM by outflow obstruction and myocardial infiltration (Table III).

Miscellaneous cardiac abnormalities (Table III) include assorted intracardiac tumors, mitral valve prolapse and aortic dilation. Strictly speaking, these latter 2 abnormalities may be regarded as valve and arterial dysplasia distinct from cardiac malformations.

It might be hypothesized that the only NF1 patients with cardiac abnormalities, especially valvar pulmonic stenosis, are those with the Watson [Watson 1967, Allanson et al., 1991] or NF1-Noonan syndrome [Sharland et al., 1992]. Most patients with NF1-Noonan syndrome do not have a CVM. Although Watson syndrome is considered an allelic variant of NF1, the molecular basis of NF1-Noonan syndrome appears diverse. Several cases of the NF1-Noonan syndrome result from an NF1 mutation [Tassabehji et al., 1993; Kayes et al., 1994; Stern et al., 1995; Colley et al., 1996], but most cases of Noonan syndrome do not have NF1. Noonan syndrome itself is a distinct disorder (with a gene locus at 12q22 in some families), and valvar pulmonic stenosis is also the most common CVM in this condition [Sharland et al., 1992]. Further cardiac overlap with NF1 is suggested by the occurrence of hypertrophic cardiomyopathy in approximately 20% of patients with Noonan syndrome [Sharland et al, 1992].

NF1 patients with large deletions are often dysmorphic and mentally retarded and two such patients have been reported with CVMs [Leppig et al., 1994; Wu et al., 1995; Wu et al., 1997; Tonsgard et al., 1997]. All of these patients (Watson syndrome, NF1-Noonan syndrome, large deletion "syndrome") are part of the NF1 population, and their association with CVMs

provides evidence that NF1 mutations can cause CVMs. In particular, the association with Watson syndrome provides evidence that NF1 can cause pulmonic stenosis, arguing for, not against, an association of NF1 and CVMs.

For this analysis of CVMs and other cardiac abnormalities in NF1 patients, we used the National Neurofibromatosis Foundation International Database (NNFFID), an international multicenter collaborative system for collecting demographic information, descriptions of signs and symptoms, basic measurements, and certain psychological information on individuals and families with NF [Friedman et al., 1993]. The NNFFID was established and is maintained by the National Neurofibromatosis Foundation. Twenty-four clinics throughout the world voluntarily enter data into the NNFFID. Complete data from over 4000 patients are currently available. Any qualified investigator can access these data for use in appropriate studies by contacting the authors.

A preliminary analysis of data from the NNFFID suggested that CVMs, especially pulmonic stenosis, are more common in NF1 [Friedman and Birch, 1998]. We present further descriptive analysis of 97 patients with verified NF1 contributed to the NNFFID between 1991 and 1998.

PATIENTS AND METHODS

As described previously [Friedman et al., 1993], the NF database collects data on 98 items and allows for serial collection. All patients in this final analysis had NF1 as defined by the NIH Consensus Conference [1988]. We tried to identify patients who may have been previously reported. We analyzed as a separate group the dysmorphic patients reported to have possible Watson or NF1-Noonan syndrome.

The aortic narrowing in patients with coarctation was characterized as either a descending thoracic juxtaductal aortic shelf usually seen in non-NF coarctation, or a fusiform or segmental hypoplasia in either the thoracic or abdominal aorta. To promote insight into the possible mechanisms and timing, cardiac abnormalities were categorized as CVMs, other non-CVM abnormalities , and peripheral vascular abnormalities [Lin and Pexeider, 1994]. A CVM was defined as a structural malformation involving intracardiac structures and/or great arteries. For this analysis, CVMs were organized by mechanistic groups using an expanded version of the classification proposed by Clark [1995]

We recorded information about the method of CVM diagnosis, ie. whether it was based clinically using auscultation, radiography and electrocardiography, or whether it was confirmed using echocardiography, catheterization, surgery or autopsy. If the NF clinician attributed a murmur to a specific CVM, the patient was said to have a clinically diagnosed CVM. However, we excluded patients with heart murmurs which were called "functional", or if an echocardiogram proved that a suspicious murmur had no anatomic basis. A left superior vena cava to the coronary sinus was also excluded as a normal variant.. In addition to CVMs, we tabulated cardiomyopathy, "dysplastic" valve defects (eg. mitral valve prolapse), intracardiac tumors and electrocardiographic abnormalities.

RESULTS

At the time of this analysis, the NNFFID contained 2550 NF1 patients (Table IV). 130 patients were submitted for review as having NF1 and a cardiac abnormality; 97 were verified as having fulfilled the NIH diagnostic criteria. Five patients with possible Watson syndrome (2), NF1-Noonan phenotype (2), or unclear diagnosis (1) were analyzed separately from the main cohort. We excluded 15 patients with "provisional" NF1 who lacked NIH criteria, 11 of whom

were very young patients (less than 10 years) with multiple café-au-lair spots and insufficient followup, evaluation or family history. Five (33%) of these young "provisional" patients had pulmonic stenosis, specified as valvar in one.

Table V lists clinical characteristics of the cohort including slight male predominance (53%) and young age (46% younger than 10 years). The Watson and NF1-Noonan patients were 6 years or younger. The diagnosis of most (69%) CVMs was either clinical or not stated. The diagnosis was confirmed by echocardiography alone or with another test (21%), catheterization alone (none), or surgery (9%).

Fifty (52%) of the 97 verified patients with NF1 had a CVM. This represents 2% (50/2550) of the patients in the NNFFID (Table VI). Excluding the 8 patients reported as having a "possible" or inadequately specified CVM, the frequency decreases to 1.6% (42/2550). Most (40/50, 80%) of the CVMs could be classified as "flow defects", in which right or left heart obstruction, or simple shunts are postulated to be the result of abnormal embryonic intracardiac hemodynamics [Clark, 1995]. Most (22/40, 44%) had pulmonic stenosis, usually specified (or presumed) as valvar. One boy from Children's Hospital in Boston with a pulmonic stenosis murmur had been reported as a patient with a large deletion [#96-665 in Wu et al., 1995].

Left heart obstruction (aortic stenosis, coarctation) was reported in 14% (7/50). Of 5 patients with "coarctation", 3 had sufficiently detailed medical records to determine that the aortic narrowing was a long fusiform narrowing, in contrast to the "typical" juxtaductal shelf seen in the general population.

In this study, there were 2 (4%) patients with tetralogy of Fallot. This can be viewed as a conotruncal CVM, part of Class I which is attributed to altered mesenchymal cell migration. There were no patients with Class IV atrioventricular canal defects which are attributed to

abnormal extracellular matrix, and no patients with looping or laterality defects. This distribution compares to the Baltimore-Washington Infant Study of newborns [Ferencz et al., 1993] in which Class II "flow" defects occurred in 64%, followed in frequency by Class I "conotruncal" (17%), Class IV "atrioventricular canal" (11%). With respect to the Baltimore-Washington Infant Study, the NF1 patients were no more likely to have a Class I defect (OR=1.4; 95% CI 0.2 – 5.6), but appeared more likely to have a Class II lesion: OR=7.3 (95% CI 5.2-10.2).

Table VII lists 26 (27%) verified NF1 patients with other cardiac abnormalities, most of whom had unspecified murmurs (15/26, 58%). There were no patients in this NNFFID cohort with HCM. Table VIII enumerates 16 (16%) patients with peripheral vascular abnormalities. In addition to 11 patients with arterial stenosis, 2 had moya-moya disease, 1 had an arteriovenous malformations, and 1 had an aneurysm of the right subclavian artery.

DISCUSSION

The participants in the NNFFID are a heterogeneous group of centers who contribute the data voluntarily. Since most are at tertiary level hospitals, the NF1 patients they see may be more likely to have serious medical problems (such as CVMs) than an unselected group of NF1 patients. On the other hand, very few of our clinics have any particular interest in cardiac disease, as demonstrated in many cases by the poor quality of data about the CVMs. We suspect that additional cases with CVMs exist among the patients in our database.

This study is the largest review of cardiac abnormalities in NF1. The overall frequency of 2% of CVMs among NF1 patients without Watson or NF1-Noonan syndrome in the NNFFID is similar to what was reported by Carey et al. [1979] (Table 1). Colley et al.'s [1996] figure of 2.0% among 453 patients was reduced to 0.7% after excluding 2 patients with Watson syndrome

and 4 patients with NF1-Noonan syndrome. This estimate of CVMs in NF1 (about 20/1000) cannot be fairly compared to the population incidence (about 5/1,000) of children ascertained to age 1 year in the Baltimore-Washington Infant Study [Ferencz et al., 1993]. The age distribution and methodology are strikingly different. It is likely that some clinically mild CVMs may have been silent during the BWIS study period, with detection beyond age 1year. Cardiovascular malformations in NF1 may have been underestimated if some of the patients excluded for incomplete NIH criteria had a CVM. There may have been an overestimate due to referral bias since patients with CVMs were more likely to be seen by specialists or would recognize NF1. Because a large proportion of the reported CVMs in this series were diagnosed clinically (28%) or not stated (41%), we cannot be certain that a CVM was present.

Perhaps as important as the predominance of Class II "flow" defects, in particular pulmonic valve stenosis, is the absence or low frequency of more complex CVMs such as laterality and looping defects, conotruncal, and atrioventricular CVMs. In particular, the paucity of conotruncal CVMs is a striking contrast to the type of CVM (double-outlet right ventricle) observed in the NF1 "knockout" mouse homozygous mutant embryos [Jacks et al., 1994]. Double outlet right ventricle is one of the conotruncal CVMs, attributed to abnormal migration of ectomesenchymal cell migration [Clark, 1995]. These are derived from the neural crest which contributes to the function of the outflow tracts [Kirby et al., 1983]. Developmental mechanisms besides neural crest migration may play a role in causing CVMs in NF1. The rarity of complex CVMs supports the notion that NF1 is not a multiple malformation syndrome; it is best viewed as a multiple dysplasia syndrome [Carey and Viskochil, 1999].

Coarctation of the aorta was a fusiform narrowing of the descending thoracic aorta in 3/5 NF1 patients. This probably reflects the recognized arteriopathy of NF1 rather than a bonafide

malformation. This study reaffirmed the association of other peripheral vascular anomalies in NF1 (Table VIII). Aside from the patients with unspecified heart murmurs, we detected mitral valve prolapse and cardiac tumor, but no patients with HCM in the NNFFID (Table II, VII). The data remain insufficient to determine if NF1 and HCM are associated. Of patients reported in the literature (Table II), none has been analyzed for either the NF1 or common HCM mutations [Coonar and McKenna, 1997].

There is no firm genotype-phenotype correlation with particular NF1 mutations. Many NF1 patients with deletions of the entire gene are often dysmorphic and intellectually limited [Leppig et al., 1994; Wu et al., 1995; Wu et al., 1997; Tonsgard et al., 1997], but these clinical features are not obligatory [Rasmussen et al., 1998]. A CVM was noted in 2 reported patients [Leppig et al, 1994; Wu et al., 1995] (Table 1). Since the NIH criteria remain the clinical "gold standard" for diagnosis, molecular analysis for NF1 was not done for this NNFFID study group.

A weakness of this study was our lack of knowledge regarding molecular analysis of the NF1, Noonan syndrome (12q in some patients), and hypertrophic cardiomyopathy mutations to patients in the NNFFID. This would better define patients with (1) Watson phenotype, (2) NF1-Noonan phenotype, and (3) NF1 and hypertrophic cardiomyopathy (though none were observed in this cohort). Another weakness was the lack of diagnostic confirmation in all patients.

To obtain more accurate information about CVMs in NF1, a multi-center study is needed involving geneticists and cardiologists to ensure accurate diagnosis of NF1 and cardiac abnormalities. Cross-sectional echocardiography could be used to evaluate stratified age groups. The natural history of pulmonic stenosis in NF1 should be studied to compare its progression (or regression) with the general population. It appears to be generally mild, and not a cause of

serious morbidity. To pursue an investigation of HCM in NF1, echocardiography could also be used to monitor for obvious hypertrophy, and subtler increased cardiac mass.

CONCLUSIONS

Among patients entered into the NNFFID, there was a higher than expected frequency of CVMs compared to newborn population estimates. The possible explanations include: 1) CVMs represent an uncommon (2%) but predictable manifestation of NF1, a hypothesis supported by the predominance of pulmonic stenosis and the rarity of other types of CVMs; 2) CVMs, especially pulmonic stenosis, and NF1 are common disorders which may occur together by chance alone. This study could not address whether: 3) CVMs occur in NF1 patients with large deletions. It seems unlikely that 4) most NF1 patients with pulmonic stenosis have either the Watson syndrome, or NF1-Noonan phenotype.

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TABLE I. Literature Review of Cardiovascular Malformations in NF1 Patients.*

Author	Year	# NF1 patients	#CVM patients	%	Age	Gender	Comment
Large series:							
Crowe et al.	1956	223	1 ASD	0.4 %	37	M	The single patient was clinically diagnosed.
Kaufman et al.	1972	NS (19 families)	6 total:				None had features of Noonan or Watson syndrome.
			5 PSV		10	M	
					53	M	Unclear if leaflets abnormal; annulus definitely small.
					4	M	May also have had thickening of aortic valve leaflets.
					3	F	Brother (1 mo): COA, ASD. No CLS to diagnose NF1. Sister (8): Clinically diagnosed VSD. 3 CLS. Brother (7): Clinically diagnosed VSD, CLS, MR.
					18	M	
			1 SVAS		6	M	Paternal uncle with NF1 and PVS.
Neiman et al.	1974	78	5 total:	6.4 %			
			Family				May represent NF1-Noonan syndrome.
			1 PSV		2 9/12	M	Brother. Also, MR, VPI.
			1 COA, BAV		4 ½	F	Sister. Also, MR, "Turner phenotype"
			1 PSV		>18	F	Mother. Also, MR, "Turner phenotype"
			Unrelated cases				
			1 VSD		9/12	M	Location of VSD NS.
			1 ASD		6	M	Location of ASD NS, probably secundum.
			(1 CHB)		5	F	Exclude since there is no underlying CVM.
Holt	1978	NS	3 NS	NS	NS	NS	Supplements preceding series of Crowe et al., and Neiman et al.
Carey et al.	1979	131 (60 families)	2 NS	1.5 %	NS	NS	
Schorry et al.	1989	78	2 NS	2.6%	NS	NS	

Colley et al.	1996	453 (235 families)	9 PS (3 PS)	2.0 (0.7%)	NS NS	NS NS	(After excluding 4 patients with NF1-Noonan syndrome and 2 patients with Watson syndrome)
Tonsgard et al.	1997	35 (406 total)	4 total: 2 PPS 1 VSD, NOS				From general NF1 cohort, 70 had features of deletions. 35 (26 families) were studied, 4 had deletions
Small series or individual case reports:							
Zoethout et al.	1964	1	1 ASV	NS	39	M	1 NF patient in a series of 126 patients with AS.
Decken and Caplan	1970	1	1 ASV	NS	NS	NS	Patient had aplasia cutis congenita
Rosenquist et al.	1970	NS	1 RVOT membrane	NS	9	F	Right ventricular hypertrophy probably secondary to RVOT, not a primary cardiomyopathy
Pernot et al.	1971	NS	3 PSV	NS	9 2 1	F F F	All 3 had CLS, none had neurofibromas. Unclear if NF1, NF1-Noonan syndrome, Watson. Fourth patient described as having lentiginosis.
Wille et al.	1980						See Table III for description. Unknown if "aortic obstruction" was valvar (ie. a CVM), or subaortic hypertrophy (ie. HCM).
Shimada et al.	1981	1	1 ECD	NS	24	M	

Leppig et al.	1994	5	1	1 ASD, NOS	5	M	All 5 patients had dysmorphic features, MR, and NF1 gene deletions.
Wu et al.	1995	4	1	1 PS murmur	16	M	All 4 patients had dysmorphic features, MR, and NF1 gene deletions.
Stoll et al.	1995	1		1 dTGA, TriAtr, PAS, ASD, VSD	birth	F	NF1 not substantiated. CLS, no NFs. Multiple anomalies (microcephaly, dysmorphic face, scoliosis, short digits), pheochromocytoma. Parents first cousins.
Fischberg et al.	1996	1		1 Vascular ring NS	birth	M	Esophageal compression due to both vascular ring and disseminated neurofibromatosis (including atrial septum and auricular appendages).

* Not included are patients with segmental or fusiform "coarctation" of the abdominal aorta, which is considered part of the NF1 vasculopathy.

Abbreviations: AS, aortic stenosis; ASD, atrial septal defect; ASsub, sub-aortic stenosis; ASV, aortic stenosis, valvar; BAV, bicuspid aortic valve; CHB, complete heart block; CLS, café au lait spots; COA, coarctation; CVM, cardiovascular malformation; dTGA, dextro-transposition of great arteries; ECD, endocardial cushion defect; F, female. M, male; MR, mental retardation; NF, neurofibromatosis; NS, not specified, not available or not applicable; PAS, pulmonary artery stenosis; PS, pulmonic stenosis; PPS, peripheral pulmonic stenosis; RVOT, right ventricular outflow tract obstruction; TriAtr, tricuspid atresia; VPI, velo-pharyngeal insufficiency; VSD, ventricular septal defect

TABLE II. Literature Review of Cardiomyopathy in NF1.

Author	Year	# patients and type of CM	Age	Gender	Location	Severity	Comment
Pung and Hirsch	1955	1	"HCM"	4	F	BVH	severe	Hypertrophic ventricles probably due to extrinsic neurofibromas.
Goodwin	1974	1 NS	NS	NS	LV	NS	Mentioned briefly in text, no details.
Gerbaux et al.	1974	1 HCM	13	M	LV	moderate	"Obstructive myocardiopathy". Described as having a Turner phenotype ("male Ullrich") and NF1. Suspect NF1-Noonan syndrome.
Elliot et al.	1976	1 HCM	44	F	LV	moderate	"IHSS"
Benotti et al.	1980	1 RCM	56	M	BVH	NS	
Wille et al.	1980	2 HCM Family					
		father	68	M	LV	severe	Probands had both "aortic and outflow obstruction" and NF1. One daughter had NF1. Seven other relatives had HCM. Likely that pedigree illustrates 2 diseases co-segregating in probands. One proband (at least) extremely dysmorphic.
		son	29	M	LV	NS	
Mercier et al.	1981	2 HCM	(<11)	NS	LV	NS	Briefly mentioned in text.
			(<11)	NS	LV	NS	Age range 7 days - 11 yrs.
Sachs et al.	1984	2 HCM	70	M	LV	severe	Post-mortem histology non-specific (without myofiber disarray).

			51	M		LV	severe	
Tillous-Borde et al.	1986	1 HCM	1 ½	F		BVH	severe	Spontaneous regression at 6 weeks. Maternal diabetes not present.
Hosokawa et al.	1986	1 HCM	___	___		LV	____	"IHSS"
Waxman et al.	1986	1 HCM	57	F		LV	NS	"IHSS"
Salvadori et al.	1986	1 HCM	38	M		LV	moderate	HCM, but not IHSS. Localized to infero-apical portion of septum and postero-lateral wall.
Schrader et al.	1986	1 HCM	58	F		LV	severe	Myocardial biopsy showed fiber disproportion and interstitial fibrosis.
Fitzpatrick and Emanuel	1988	2 HCM Family						Seven other family members with NF1 without cardiac disease.
		sister	46	F		LV	severe	
		Brother42	M		LV		severe	No one with HCM alone.

Abbreviations: BVH, biventricular; HCM, hypertrophic cardiomyopathy; IHSS, idiopathic subaortic stenosis; LV, left ventricle; NS, not specified; RCM, restrictive cardiomyopathy

TABLE III. Literature Review of Intracardiac Tumors and Other Cardiac Abnormalities in NF1

Author	Year	# patients	Age	Gender	Comment
Intracardiac Tumors:					
Frankiewicz	1973	1	16	M	Tumor: left atrial neurofibroma
McAllister & Fenoglio	1978	2	NS	NS	Tumor: neurofibroma. Unclear which 2 of the 3 reported patients with neurofibroma of the pericardium (1) and myocardium (2) had NF1.
Mata et al.	1981	1	27	F	Tumor: myocardial rhabdomyosarcoma
Takeda et al.	1982	1	NS	NS	Tumor: intrapericardial (histology not stated)
Fischberg et al.	1996	1			Tumor: atrial septum and appendages (in addition to vascular ring, vide supra)
Noubani et al.	1997	1	10	F	Tumor: infiltration of heart from mediastinal neurofibroma. (mitral valve disease secondary to rheumatic fever.)
Miscellaneous:					
Etches and Pickering	1978	1	11	F	MVP clinically, not confirmed.
Halper and Factor	1984	1	38	M	Nonocclusive coronary artery thickening
Bensaid et al.	1986	1	67	M	MVP by echocardiography.

Scotto di Uccio et al.	1988	1	52	M	MVP by echocardiography.
Kandarpa et al.	1988	1	30	M	Coronary aneurysm and myocardial infarction.
Uren et al.	1988	1	28	M	Left atrial wall aneurysm.
Nogami et al.	1991	2	52	F	Coronary artery spasm, "myocarditis", apical cardiac sympathetic denervation,.
			50	F	Right ventricle myofiber disarrary with interstitial fibrosis.
Kaplan and Rosenblatt (Family A, proband)	1985	1	17	M	Aortic dilation, AR, MVP. Patient thought to have NF1-Noonan phenotype.
restudied by Leppig et al. (UWA 169-1):	1997				Later, found to have microdeletion of NF1.
Wu et al	1997	1	45	M	MVP. Patient is the father of 15 yo male, both of whom had NF1 deletions.

Abbreviations: AR, aortic regurgitation; MVP, mitral valve prolapse

TABLE IV. Summary of NF1 Patients.

	Number patients		
Total number of NF1 patients in the NNFFID, 1991 - 1998	2550		
Number with verified NF1 (NIH criteria)	2323		
Total number patients submitted as having a cardiac abnormality	130		
Verified cardiac diagnosis, and NF1 (NIH criteria)		97	
Cardiovascular malformation (Table VI)			50
Other cardiac abnormality (Table VII)			26
Peripheral vascular abnormality (Table VIII)			16
Watson syndrome or NF1-Noonan phenotype			5
Excluded		33	
NF1 diagnosis incomplete, "provisional"			15
Other			8
Murmur, normal echocardiogram (CVM ruled out)			3
Left superior vena cava			2
LEOPARD syndrome			1
No cardiac diagnosis			2

TABLE V. Clinical Characteristics of 97 Verified NF1 Patients.

	Total	CVM	Other cardiac	Peripheral	Watson/ NF-NS
Total	97 (100%)	50 (52%)	26 (27%)	16 (17%)	5 (5%)
Gender:					
Female	36 (37%)	20	11	8	3
Male	51 (53%)	30	15	8	2
Age:					
≤10 years	45 (46%)	27	11	7	5
11-20 years	24 (25%)	14	9	4	0
≥21 years	18 (19%)	9	6	5	0
Method of diagnosing the cardiac abnormality:					
Clinical	27 (28%)	6	18	2	1
Echocardiography	14 (14%)	6	5	0	3
Echocardiography + another test	7 (7%)	6	0	0	1
Catheterization	0	0	0	0	0
Surgery	9 (9%)	3	0	6	0
Autopsy	0	0	0	0	0
Not stated	40 (41%)	29	3	8	0

Abbreviations: CVM, cardiovascular malformation; NF-NS; neurofibromatosis-Noonan syndrome

TABLE VI. Frequency and Type of Cardiovascular Malformations in 50 NF1 Patients.

	NF1 patients fulfilling NIH criteria				Watson syndrome / NF1-Noonan syndrome				
	Number of patients				Number of patients				
Clark Class	Conf*	Unconf	Total	(%)	Conf*	Unconf	Total	(%)	BWIS
I "Conotruncal"	**1**	**1**	**2/50**	**(4%)**					**(17%)**
Tetralogy of Fallot	1	1	2						
II "Flow"	**14**	**26**	**40/50**	**(80%)**					**(64%)**
Right heart, total	7	14	22	(44%)					(11%)
Pulmonic stenosis, total	7	15	22		3	0	3/5	(60%)	
valvar	5	2	7		2				
peripheral	0	1	1						
NS, presumed valvar	2	10	12						
+ VSD	0	1	1		1				
+ ASD	0	1	1						
Left heart, total	5	2	7	(14%)					(8%)
Aortic stenosis, total	1	1	2						
valvar	1	0	1						
NS (presumed valvar)	0	1	1						

Coarctation	4	1	5			
Simple shunts		2	9	11	(22%)	(42%)
ASD, NS, presumed secundum		0	4	4		
VSD, total		1	5	6		
NS		0	4	4		
+ SVPS + DC RV		1	0	1		
+ ASD		0	1	1		
PDA		1	0	1		
Possible, NS, or inadequately specified				8/50	(16%)	

* Confirmed by two-dimensional echocardiography, catheterization, or surgical observation

Abbreviations: ARSCA, aberrant right subclavian artery; ASD, atrial septal defect; BWIS, Baltimore-Washington Infant Study Group; DC RV, double chambered right ventricle; NS, not specified; PDA, patent ductus arteriosus; PS, pulmonic stenosis; SVPS, supra-valvar pulmonic stenosis; VSD, ventricular septal defect

TABLE VII. Other Cardiac Abnormalities in 26 Patients with NF1.

Abnormality	# patients
Cardiomyopathy	0
Murmur, no echocardiogram (CVM cannot be ruled out)	15
Mitral valve prolapse +/- mitral regurgitation	5
Tumor	
possible myxoma (possible cardiac neurofibroma)	1
Electrocardiogram abnormalty	
first degree heart block	2
"changes of left ventricular myocardium"	3

TABLE VIII. Peripheral Vascular Abnormalities in 16 Patients with NF1.

Vascular Abnormality	# Patients	4 Additional Patients with a CVM (Table VI) + peripheral vascular abnormality
Renal artery stenosis	9	1 Pulmonic stenosis, valvar + renal and femoral artery stenosis 1 Pulmonic stenosis, not specified + renal artery stenosis
Subclavian artery stenosis	1	
Middle cerebral artery stenosis	1	
Moya moya disease	2	
Arteriovenous malformations left temporal lobe	1	
Aneurysm right subclavian artery	1	1 "Multiple CHD", not specified + splenic artery aneurysm
Possible, "bruits"	1	1 Pulmonic stenosis, not specified + unspecified stenosis

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November, 1999

John Opitz, MD
Editor
American Journal of Medical Genetics
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Re: Cardiovascular Malformations and Other Cardiac Abnormalities in Neurofibromatosis 1

Dear John,

Please consider this manuscript for publication in the American Journal of Medical Genetics. This paper is a descriptive review of patients submitted to the National NF Foundation International Database.

Sincerely,

Angela E. Lin, MD
Clinical Geneticist

Mortality associated with neurofibromatosis 1 in the United States from 1983 to 1995: an analysis using data from death certificates.

S.A. Rasmussen[1], Q.H. Yang[1], J.M. Friedman[2]

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Neurofibromatosis 1 (NF1) is one of the most common autosomal dominant disorders, occurring in 1 in 3,000 individuals; however, information regarding associated mortality is limited. For this study, we used Multiple-Cause Mortality Files, compiled by the National Center for Health Statistics from US death certificates, for the years 1983 to 1995. These files included International Classification of Diseases, Ninth Revision (ICD9), codes for the underlying cause of death and up to 20 other conditions listed on the death certificate. We selected all cases listing the code for neurofibromatosis. Since this code could also include neurofibromatosis 2 (NF2), which is much less prevalent but usually more severe than NF1, we excluded cases with codes for sensorineural hearing loss or benign neoplasms of the cranial nerves or meninges as probable NF2 cases.

3,253 presumed NF1 cases were identified among over 28 million deaths, a prevalence of 1 in 8,600, suggesting under ascertainment of NF1 in this population. Mean and median ages of death for NF1 cases were 54.5 and 59 years, respectively, compared to 69.8 and 74 years in the general population, confirming previous findings of a 15-year decrease in life expectancy among those with NF1. Results of proportionate mortality ratio (PMR) analyses showed that decedents with NF1 were 38 times more likely (PMR=37.9; 95% CI=33.7-42.4) to have a malignant connective or other soft tissue neoplasm listed on their death certificate, compared to those without NF1. Overall, decedents with NF1 were only 1.2 times more likely (PMR=1.2; 95%CI=1.15-1.31) to have a malignant neoplasm than those without NF1.

Since previous NF1 mortality studies have suggested that hypertension may be related to mortality, we evaluated hypertensive diseases; these were not more likely to be listed on death certificates of those with NF1 than of those without NF1 (PMR=1.1; 95%CI=0.97-1.28). This study provides population-based mortality data useful to clinicians caring for patients with NF1.

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Logistic Regressive Models of Neurofibromatosis 1 (NF1) Features

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Most NF1 patients never develop the severe features of the disorder, but the variable expressivity of NF1 prevents prediction of which patients will develop such features. We have shown previously that several statistically significant associations exist between pair-wise combinations of NF1 features among age-stratified probands. We now extend the analysis to interactions of several different features at once.

The National Neurofibromatosis Foundation International Database (NFDB) contains extensive cross-sectional information on 2,797 probands affected with NF1. Twelve of the most common or important clinical features of NF1 were selected. They include intertriginous freckling, discrete and plexiform neurofibromas, Lisch nodules, optic gliomas, and other neoplasms.

Logistic regressive models allow for binary or polytomous dependent variables and, binary, polytomous or continuous covariates. The logit of each of the 12 features analysed was set as the output variable in 12 different models. Univariate analyses were used to screen the features for potential main effects for the multivariate analyses. NF1 is a progressive disorder and many of its features exhibit a different relationship with age. Therefore, age was controlled as precisely as possible, as a continuous covariate. Following the fit of the multivariate model, the importance of each variable was assessed. Significant terms were used to refit the model and interaction terms among the explanatory variables were considered.

Fitted models always perform in an optimistic manner on the developmental data set. Therefore, a random subsample of the NFDB subjects was excluded, models were developed based on the remaining subjects, then the models were tested in the originally excluded subjects.

The results revealed several significant reproducible interactions. For example, Lisch nodules were found to be 8.7 times more frequent in subjects with intertriginous freckling, discrete neurofibromas and neoplasms than in subjects who lacked these features. Optic glioma occurred 14 times more often in subjects with plexiform neurofibromas and neoplasms than in subjects who lacked these features. Similar interactions have been observed for the occurrence of the other modeled features.

These findings demonstrate that some patients are far more likely than others to develop certain features of NF1. This suggests that fundamental biological differences exist between subjects who differ by particular constellations of features. The logistic regressive models may be useful for the development of age- and sex-specific risks for complications of NF1 in clinically defined sub-groups of NF1 patients.

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